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ISOLATION OF *Salmonella* spp. AND OTHER MEMBERS OF *Enterobacteriaceae* FROM HORSE MACKEREL (*Trachurus trachurus*), SOLD IN PUBLIC MARKETS OF ISTANBUL, TURKEY

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Abstract:

Aim of this investigation is to provide the general information about prevalence of members of *Enterobacteriaceae* in horse mackerel (*Trachurus trachurus*) sold in open public markets in Istanbul, Turkey. Horse mackerel were randomly selected and collected in warm and cold seasons of the year. Purchased samples were kept in sterile insulated bags with ice and transported to the laboratory for microbiological analyses. *Citrobacter* spp. showed highest prevalence (45.56%), followed by *Proteus mirabilis* (22.62%), *Proteus vulgaris* (9.17%) and *Escherichia coli* (7.64%). The other isolates were *Shigella sonnei* (4.28%), *Shigella dysenteriae* (3.36%), *Salmonella* Typhimurium (1.83%), *Salmonella* Paratyphi A (1.52%), *Klebsiella pneumoniae* (1.22%), *Klebsiella oxytoca* (1.22%), *Enterobacter aerogenes* (0.91%) and *Enterobacter cloacae* (0.61%). The percentages of the isolates, obtained in spring and autumn, were also compared. The *Citrobacter* spp., *P. mirabilis* and *P. vulgaris* were the dominantly isolated species, during spring season. *P. mirabilis* and

Citrobacter spp. were also dominant in autumn. However, numbers and percentages of isolated pathogens (*E. coli*, *S. sonnei*, *S. Dysenteriae*, *S. Typhimurium* and *S. Paratyphi A*) were much more in autumn than that of spring. Since samples are sold without any chilling treatment in public markets, air temperature might be the reason of higher pathogen isolation in autumn. It is essential to implement cold chain as well as to prevent secondary contamination and to improve quality control.

Keywords: *Salmonella*, *Enterobacteriaceae*, Pathogen, Fish, Contamination, Public market

Introduction

The members of *Enterobacteriaceae* family are widespread in the environment and their natural habitat is gastrointestinal tract of warm-blooded animals. They may play an important role in the fish spoilage and some bacterial species of this family are very pathogenic (Guiraud, 2003; Shabarath et al., 2007; Lopez Da Silva et al., 2010). Pathogens such as *Salmonella* spp., *Shigella* spp. and certain *E. coli* can cause severe diarrhea (Lindberg et al., 1998). Especially *Salmonella* is dominating intestinal tract of animals (birds, reptiles, and farm animals) and humans. *Salmonella* species are extensively distributed in nature (water reservoirs, coastal water contaminated with human or animal feces) and causing outbreaks worldwide. According to Centers for Disease Control and Prevention (CDCP), almost 400 persons die every year with acute Salmonellosis. This bacterium has been isolated from fish and other seafood (Jay, 2000a; Huss et al., 2004; CDCP, 2010). It may be transferred to the seafood due to the poor hygienic conditions during transportation and marketing (Temiz, 1998). Prevalence of *Salmonella* and some bacterial species, belonging to the *Enterobacteriaceae* family, have been studied in different regions of the world and health risks were evaluated. Microbiological quality of seafood in Croatia (Topic Popovic et al., 2010), fish in Khartoum, Sudan (Yagoub, 2009), fish and crustaceans in Coimbatore, India (Hatha and Lakshmanaperumalsamy 1997), shrimp in India (Hatha et al., 1998; Jonnalagadda and Bhat, 2004), seafood in Greece (Papadopoulou et al., 2007), cooked shellfish in UK (Sagoo et al., 2007), hygiene conditions of wholesale fishmarket in Istanbul (Ucok, 2003), microbiological quality of stuffed mussel in Istanbul (Bingol et al., 2008), determination of pathogen microorganisms in seafood in Istanbul (Ucok Alakavuk, 2009) and quality of fish from retail markets in Istanbul (Mol and Tosun 2011), were studied.

Public markets are the main suppliers of fish and other seafood to the public. However, fish and other seafood are sold in Turkish public markets without chilling. Since high environmental temperature is the most important reason to encourage bacterial growth (Jay, 2000b) warmer seasons are very risky for the microbial safety of fish, displaying on the counter without any chilling treatment. On the other hand, air temperature in Istanbul City is between -2 to 5.7°C in winter, 5.5 to 17.06°C in spring, and 7.2 to 19.36°C in autumn (Turkish

State Meteorological Service (TSMS), 2013). Then, the warmer seasons (spring and autumn) are the most risky periods, regarding the growth of *Salmonella* and other members of *Enterobacteriaceae* on fish, sold in public markets without chilling. Horse mackerel (*Trachurus trachurus*), a common fish in Turkish waters, having an annual catching value of 12213.2 ton (TUIK, 2015), and this catch has been offered for domestic market as well as export market. Since it is very popular for Turkish consumers, horse mackerel is one of the top selling fish in public markets. The aim of this study, is the determination of *Salmonella* and members of *Enterobacteriaceae* on horse mackerel, sold in public markets in Istanbul.

Materials and Methods

Sample collection

The average air temperatures were 13.7°C in spring and 15.75°C in autumn, during this study. The highest air temperatures in spring and in autumn were 22.2°C, and 26.0°C, respectively. Horse mackerel samples (16.03 ±5.91g, 11.76 ±1.24cm) were randomly purchased from the public markets in 31 districts of Istanbul (Figure 1) twice in spring and twice in autumn. Therefore, sampling was performed 124 times. Thirty individuals of horse mackerels were purchased from each public market (31 markets). Therefore, a total of 3720 fish samples (30 individual's x 124 times) were used in this investigation. Samples were collected from public markets between the hours of 09:00-12:00, they were kept in sterile insulated bags, iced and transported to the laboratory in less than 3 hours.

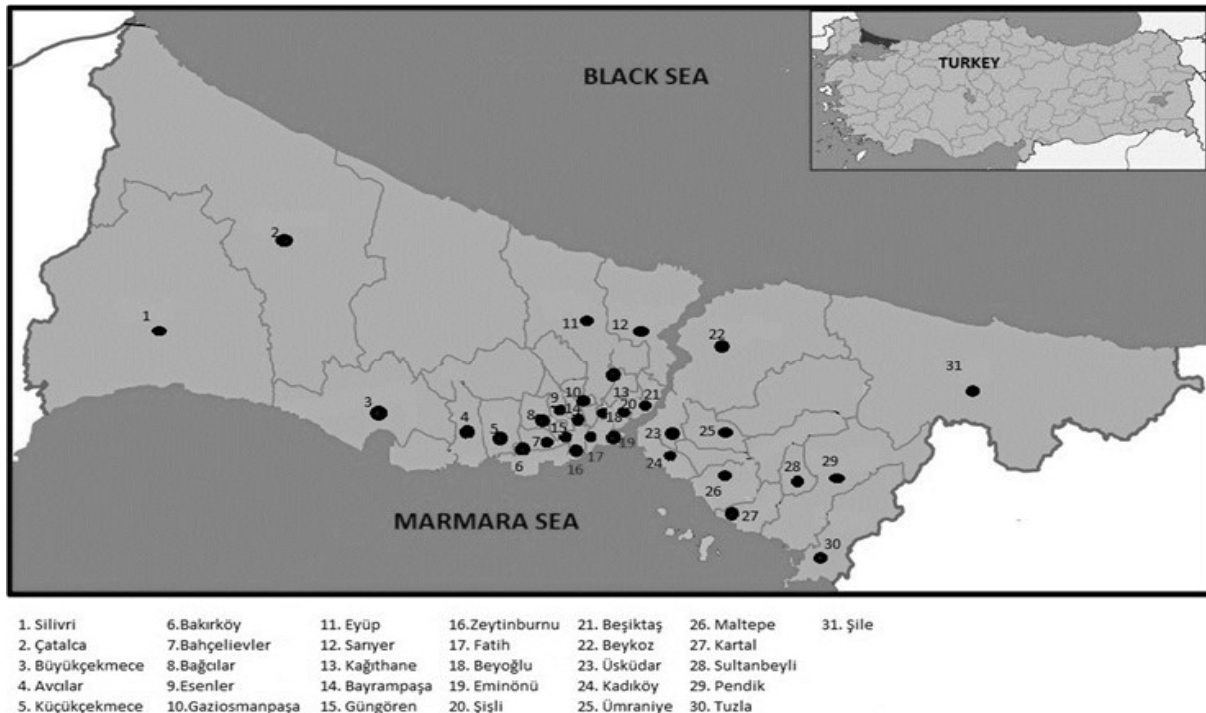
Microbiological examination

The samples, purchased from each of 31 public markets were homogenized separately. Then, 25 g of homogenate was added to the enrichment broth (225 mL Lactose Broth, Merck, 1.07661.0500), and incubated at 37°C for 24-48 hours. 0.1 mL homogenate from enrichment broth was transferred into the 10 mL of Rappaport-Vassiliadis Broth (Merck 1.07700.500) and then incubated at 42°C for 24 hours for the selective enrichment. At the same time 1 mL homogenate from lactose broth was transferred in to 10 mL Tetrathionate Broth (Merck 1.05285.0500) and then incubated for 24 hours at 43°C. After the incubation, a loopful of broth cultures were streaked onto XLT4 Agar (Merck 1.13919.0500) and Bismut Sulfite Agar

(Merck 1.05418.0500). Plates were incubated at 35°C for 48 h. After incubation, black zone all colonies (XLT4) and black center with metallic sheen colonies (BSA) streaked on TSI (Triple Sugar Iron) Agar (Merck 1.03915.0500) and LI (Lysine Iron) Agar (Merck 1.11640.0500). TSI and LI slant agar were incubated at 35°C for 48 h. and at 35°C for 16-24 h. respectively. All slant agar tubes were tested biochemically (motility, urea, H₂S,

MR-VP, indole, ONPG, lactose, sucrose, mannitol, malonate, sitrat) after incubation. All salmonella cultures were confirmed by using Salmonella Latex Kid (OXOID FT 0203,0204, 0205) after biochemical tests. (Andrews et al., 2007). *Enterobacteriaceae* colonies were identified by using a scheme 'Caracteristique des principales *Enterobacteries*' (Guiraud, 2003).

Figure1. Public markets in 31 districts of Istanbul



Results and Discussion

Some members of *Enterobacteriaceae* such as *Salmonella sp.*, *E. coli*, *Proteus sp.* and *Klebsiella sp.* may cause serious infections. Therefore, monitoring of *Enterobacteriaceae* in seafood is important for public health (Al-Mutairi, 2011). Out of 124 sampling 77 (62.10%) showed a positive growth for *Enterobacteriaceae* (Table 1). Percentages of isolates, from *Enterobacteriaceae* positive samples were 69.72% in spring and 30.27% in autumn (Table 2). The isolated species were *S. Typhimurium*, *S. Paratyphi A*, *E. coli*, *S. dysenteriae*, *S. sonnei*, *Citrobacter spp.*, *P. mirabilis*, *P. vulgaris*, *K. pneumonia*, *K. oxytoca*, *E. aerogenes* and *E. cloacae*. The numbers and percentages of bacterial species, isolated from horse mackerels during in spring and autumn were shown in Table 3. These organisms were isolated from shrimp in India (Jonnalagadda and Bhat, 2004), seafoods in

Greece (Papadopoulou et al., 2007), fresh and frozen seafoods in Croatia (Topic Popovic et al., 2010), fresh fish in Sudan (Goja, 2013), similarly. Some of the potential pathogenic microorganisms (*Citrobacter spp.*, *P. mirabilis* and *P. vulgaris*) were isolated during spring season. *Citrobacter spp.* (42.81%), *P. mirabilis* (14.67%) and *P. vulgaris* (8.25%) were mostly determined in spring. *P. mirabilis* (7.95%) and *Citrobacter spp.* (2.85%) were also determined in autumn. However, pathogenic species such as *S. Typhimurium* (1.83%), *S. Paratyphi A* (1.52%), *E. coli* (7.33%), *S. dysenteriae* (3.05%), *S. sonnei* (3.66%) were dominant in autumn (Table 3). Therefore, even the percentage of isolates was higher in spring; autumn was considered as a more risky season. The high percentage of pathogenic bacteria in autumn might be attributed to the suitability of air temperature for their survival and multiplication. Absence of

chilling treatment in public markets allows bacterial growth as well. Yagoub, (2009) reported the highest presence (66%) of *Enterobacteriaceae* from raw fish from a market in autumn, similarly. They have isolated some highly pathogenic agents such as *Salmonella* spp., *Shigella* spp., and the pathogenic *E. coli*. They have mentioned about the possible public health risks, due to this microbial activity in the autumn. Maintenance of sanitation and temperature control are very important for microbial quality, especially in warmer seasons due to the high ambient temperature (Mol and Tosun, 2011).

Salmonella spp. are one of the most important reasons of gastrointestinal diseases. Maintenance of personal hygiene is needed to prevent transmitting of these bacteria, since gastrointestinal tract is the main reservoir of them (Huss et al., 2004). According to European Union (EU) regulations on 25 g of seafood must not contain *Salmonella* sp. (Forsythe, 2010a). In this study, samples were free of

S. Typhimurium and *S. Paratyphi A* in spring season (Table 3). However, 1.83% of the samples contained *S. Typhimurium* and 1.52% of them were *S. Paratyphi A* positive in autumn. Likewise, 2.8% of raw seafood were *Salmonella* positive, according to Heinitz et al., (2000). Brands et al., (2005) reported a higher percentage of isolated *Salmonella* from oysters in the summer (13.4%) than winter (1.6%), similarly. The prevalence of *Salmonella* in shrimp have been reported as 11% and 53%, from Hyderabad, India (Jonnalagadda and Bhat, 2004) and Thailand (Minami et al., 2010). Likewise, 14.25% of the fish, from retail markets in Coimbatore, India and the 15% shellfish from markets in Ho Chi Minh City, Vietnam have been reported as positive for *Salmonella* (Hatha and Lakshmanaperumalsamy, 1997; Hao Van et al., 2007). It may be concluded that, higher air temperature encourages the growth of *Salmonella*. In this study, the presence of a higher amount of *S. Typhimurium* and *S. Paratyphi A* in autumn season, might be the result of higher ambient temperature.

Table 1. Numbers and percentages of samplings, showing a positive growth of *Enterobacteriaceae*

| | | Positive growth of <i>Enterobacteriaceae</i> | Percentage of occurrence (%) |
|-----------------|---------------|--|------------------------------|
| Sampling season | Autumn (n=62) | 29 | 46.77 |
| | Spring (n=62) | 48 | 77.42 |
| TOTAL (n=124) | | 77 | 62.10 |

Table 2. Numbers and percentages of isolates, from *Enterobacteriaceae* positive samples

| Isolated organism | Total | Autumn | | Spring | |
|---------------------------|-------|--------------------|----------------|--------------------|----------------|
| | | Number of isolates | Percentage (%) | Number of isolates | Percentage (%) |
| <i>Enterobacteriaceae</i> | 327 | 99 | 30.27 | 228 | 69.72 |

Table 3. Numbers and percentages of bacterial species, isolated from horse mackerel samples

| Isolated bacterial species | Autumn | | Spring | | Total | |
|-------------------------------|--------------------|----------------|--------------------|----------------|--------------------|----------------|
| | Number of isolates | Percentage (%) | Number of isolates | Percentage (%) | Number of isolates | Percentage (%) |
| <i>Escherichia coli</i> | 24 | 7.33 | 1 | 0.3 | 25 | 7.64 |
| <i>Salmonella typhimurium</i> | 6 | 1.83 | ND | - | 6 | 1.83 |
| <i>Salmonella paratyphi A</i> | 5 | 1.52 | ND | - | 5 | 1.52 |
| <i>Shigella dysenteriae</i> | 10 | 3.05 | 1 | 0.3 | 11 | 3.36 |
| <i>Shigella sonnei</i> | 12 | 3.66 | 2 | 0.61 | 14 | 4.28 |
| <i>Proteus mirabilis</i> | 26 | 7.95 | 48 | 14.67 | 74 | 22.62 |
| <i>Proteus vulgaris</i> | 3 | 0.91 | 27 | 8.25 | 30 | 9.17 |
| <i>Enterobacter cloacae</i> | 2 | 0.61 | ND | - | 2 | 0.61 |
| <i>Enterobacter aerogenes</i> | 1 | 0.3 | 2 | 0.61 | 3 | 0.91 |
| <i>Klebsiella oxytoca</i> | 1 | 0.3 | 3 | 0.91 | 4 | 1.22 |
| <i>Klebsiella pneumoniae</i> | ND | - | 4 | 1.22 | 4 | 1.22 |
| <i>Citrobacter spp.</i> | 9 | 2.85 | 140 | 42.81 | 149 | 45.56 |

ND: Not detected

E. coli has been found in the intestinal flora of humans and warm-blooded animals. This microorganism may transfer to the foods due to the poor hygienic conditions, cross contamination or contaminated water (Huss et al., 2004). In the present study, *E. coli* was one of the dominant bacterial species in autumn; and the percentage of isolated *E. coli* was higher (7.33%) in autumn than that of the samples collected in spring (0.3%) (Table 3). High prevalence of *E. coli* in fish, from local retailers in Greece has been reported by Papadopoulos et al., (2007). Similarly, 6.7% of the fish and seafood samples, from wholesale and retail markets in Seoul, Korea; have been reported as positive (Ryu et al., 2012). Lopez Da Silva et al., (2010) isolated *E. coli* from 10% of fish, from street markets in Sao Paulo, Brazil. On the other hand, the dominant isolate in the fish, from public market in Khartoum, Sudan (Yagoub, 2009) and in the catfish, from different markets in Nigeria (Adebayo-Tayo et al., 2012) has been reported as *E. coli*. Likewise, 48.95% of fish and fish products from Punjab, India (Gupta et al., 2013); and 38.8% of fish and shellfish Mangalore, India (Kumar et al. 2005) have been reported as *E. coli* positive. Ananchaipattana et al., (2012) also reported the highest presence (70%) of *E. coli* from seafood from retail markets (Open and supermarket) in Thailand. It was seen that; a higher isolation rates of *E. coli* have been reported in warmer climate countries.

Shigella species are the natural inhabitants of the intestinal tract of humans (Ray and Bhunia 2008). Poor personal hygiene is one of the main reasons of *Shigella* contamination, and generally the largest number of *Shigella* outbreaks have been seen in the warmer months (Huss et al., 2004). In this study, *S. dysenteriae* (3.05%) and *S. sonnei* (3.66%) were mostly seen in autumn (Table 3). Out of 150 collected samples from a fish market in Sudan, 2.2% showed positive isolation of *Shigella spp.* (Yagoub, 2009). Likewise; David et al., (2009) reported a high prevalence (39.7%) of *Shigella spp.* in fish, harvested from Kenya. Regarding the higher occurrence of *Shigella spp.* in middle climate countries; our results were associated with the higher ambient temperature in autumn.

Proteus species have a high proteolytic activity and they may easily spoil seafood, stored above refrigeration temperatures. *Proteus vulgaris* and *Proteus mirabilis* may cause diarrhea when they infect foods. Poor hygiene rules are the main cause of contamination (Ayhan, 2000). In this study, the percentages of *Proteus mirabilis*, isolated from horse mackerel were 14.67% in spring, and 7.95% in autumn (Table 3). As to *Proteus vulgaris*, the percentages of isolation in spring and autumn were 8.25% and 0.91%, respectively. The isolation of *Proteus spp.* from fish has also been reported in former studies (David et al., 2009; Lopez-Sabater et al., 1996). Yagoub, (2009), isolated *Proteus spp.* in fish with an incidence of 10.2%. The prevalence

of *Proteus* spp. in shrimp from wholesale markets has been reported as 25% by Jonnalagadda and Bhat, (2004).

Escherichia, *Klebsiella*, *Enterobacter* and *Citrobacter* are the “coliforms”. Coliforms are naturally present in the gastrointestinal tract of man and animals. Isolation of these microorganism groups indicates fecal contamination (Forsythe, 2010b). In our study, samples were free of *Klebsiella pneumoniae* in autumn season. *Klebsiella pneumoniae* was isolated 1.22% in spring season. *Klebsiella oxytoca* was isolated 0.3% in autumn and 0.91% in spring (Table 3). The prevalence of *Klebsiella* spp. in shrimps, imported from Thailand has been reported by Navaz et al. (2012). *Klebsiella pneumoniae* was isolated from blue crab (Reinhard et. al. 1996) and tropical marine fish (Singh et al., 2012), in former studies. *Enterobacter cloacae* was isolated 0.61 % in autumn. The samples were free of *Enterobacter cloacae* in spring season. *Enterobacter aerogenes* isolated from horse mackerel samples were 0.61% in spring, and 0.3 % in autumn (Table 3). The presences of *Klebsiella* spp. and *Enterobacter* spp. in fish have been reported as 2.4%, and 10.8% respectively (Yagoub, 2009). Adededi and Ibrahim, 2011, and David et al., 2009 have also reported contamination of shrimp and fish with *E. aerogenes*. The present study, the percentage of isolated *Citrobacter* spp. was higher (42.81%) in spring than that of the samples collected in autumn (2.8%) (Table 3). Contamination of seafood with *Citrobacter freundii* (Papadopoulou et al., 2007) and fish with *Citrobacter* spp. (Yagoub, 2009) have reported in former studies. *Citrobacter* spp. are widely distributed in the environment (soil, water, plants). These bacteria can be found in the intestinal flora of humans (Baylis et al., 2011). High prevalence reason for *Citrobacter* spp. may be poor personal hygiene and cross contamination.

Conclusion

It was concluded that horse mackerel, selling without any chilling treatment in the public markets of Istanbul might be a source of primary pathogens and opportunistic pathogens. The opportunistic pathogens were dominantly isolated during spring, while primary pathogens were dominant in autumn. Air temperature was regarded as an important reason of pathogen growth. Microbial contamination of fish selling in public markets can be prevented by good hygiene practice. Results of this study may be helpful to realize inadequacies and may conduce to improve selling conditions.

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