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The Occurrence of *Salmonella* in Various Marine Environments in Turkey

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1. Introduction

The occurrence and survival of enteric bacteria in marine ecosystems has been of interest to microbial ecology, sustainable usage of aquatic products, and the health of humans and the ecosystem (Barcina et al., 1986; Borrego and Figueras, 1997; Dionisio et al., 2000). Therefore, it is interesting to know and evaluate environmental factors that influence the occurrence of indicator bacteria and *Salmonella* spp. regarding sustainable and economical usage of aquatic products, ecosystem and human health.

The majority of bacteria present in domestic wastewater are comprised of saprophyte bacteria of faecal or terrestrial origin and pathogen bacteria such as *Salmonella, Shigella, Brucella*, *Mycobacterium*, *Escherichia coli*, *Leptospira, Campylobacter* and *Vibrio*. Furthermore, *Adenovirüs, Reovirüs, Rotavirüs* and *Hepatit* viruses as well as prozoons such as *Entamoeba histolytica, Giardia lamblia,* and *Cryptosporidium* may contaminate the sea by means of wastewater (Lynch and Hobbie 1988, Westwood 1994, Black 1996.)

Salmonella spp., one of the pathogenic bacteria which enter the sea environment as a result of anthropologic influences and particularly recreational use in coastal areas, continues to be a problem with regard to public health.

In order to define the source of *Salmonella* spp., contamination strains isolated from seawater and rivers were studied by molecular marker methods. Their properties were compared with those of strains originating from possible sources of contamination such as sewage from humans, cattle, and treated sewage water used in watering plants (Graeber et al., 1995).

The perforation of *Salmonella* spp. into sea water is not only from terrestrial originated wastewater but also from ships' ballast water which is imported to and exported from ships to maintain their balance.

The movements of ballast waters, from one continent to another by ships, create a global distribution mechanism for pathogenic and antibiotic-resistant forms and it may be significant in the worldwide distribution of microorganisms, as well as for the epidemiology of waterborne diseases affecting plants and animals (Ruiz et al., 2000). At the same time, most of the pathogens sourcing from sewage have been found to be present in shellfish. Particularly in production areas which are under the heavy influence of contamination, the most frequently found pathogen in shellfish is *Salmonella* spp.

1.1 The presence of *Salmonella* spp. and its relationship with primary hydrographic parameters

The presence of *Salmonella* and its relationship with primary hydrographic parameters (temperature, salinity, and dissolved oxygen) and indicator organisms in various marine environments were previously partly documented. It is known that the results of microbiological analysis were influenced by the dynamic structure of the aquatic environments. For instance, estuaries, lagoons, coastal and offshore environments are under variable environmental influences from each other. The hydrodynamic parameters of the estuary, in particular the flow rate, salinity gradient, and tidal cycles, were reported to be possible different relations between faecal-bacterial indicators and pathogens (Mill et al., 2006). Water temperature was positively associated with total *Salmonella* spp. levels. Bradd et. all (2009) reported that the levels of *Salmonella* spp. were correlated with average daily watershed rainfall for the 1 and 2 days preceding each sample collection. Similarly, environmental factors such as seasonal rainfall, salinity, and temperature were also correlated with *Salmonella* spp. abundance and diversity in the environment. (Bradd et. all 2009, Dionisio et al., 2000, Lemarchand and Lebaron, 2003; Martinez-Urtaza et al., 2004).

1.2 The presence of *Salmonella* spp. and its relationship with economically important aquatic products

The presence of *Salmonella* spp. and its relationship with aquatic products with respect to food health is one of the important headlines of this issue. Providing quality safety of aquatic products from their catching to their marketing to consumers has great importance in terms of human health as well as economical and ecological aspects.

Shellfish are filter-feeding organisms and because their power of movement is limited, they feed on the organic substances which the sea brings. They can reflect bacterial changes around them because they are capable of accumulating bacteria in high concentrations and the accumulation rate can change depending on microbial species. It was reported that *Chamalea gallina* can accumulate *S. typhimurium*, *E. coli, Vibrio parahaemolyticus, Aeromonas hydrophyla, Streptococcus faecalis,* and *Staphylococcus aureus* in the first six hours in laboratory conditions (Martinez et al., 1991). Nunes and Parsons (1998) reported that feeding oysters filter the surrounding water at a rate of 2 to 5 liter/hour eventually assimilating all the biotic and abiotic contaminants present in their environment. Due to the sensitivity of organisms and accumulation of environmental contamination, more bacterial contamination can be found in mussels than in the sea samples surrounding them. Because of these characteristics, shellfish have been accepted as bioindicators for detecting bacterial contamination in marine environments.

Salmonella spp. infections are one of the primary illnesses caused by the consumption of mussels. Bacterial pollution levels, associated with anthropological factors, are related to the occurrence of pathogenic bacteria in marine environments. *S. typhi* was isolated frequently in bivalve molluscs which were caught from a contaminated sea region. *Salmonella* spp. is one of the most important causes of human gastrointestinal diseases worldwide. Inal et al. (1979) have isolated *S. typhi* in shellfish taken from regions contaminated by slaughterhouse wastewater on the coast of the Aegean Sea, Turkey.

For these reasons, the consumption of shellfish has been generally associated with food-related infective diseases (Cook et al., 2001, Jose 1996). Food borne hazards are still of great concern for human health. Particularly the risks connected with shellfish and seafood consumption continue to be important both in developing and developed countries despite

the advances in technology, changes in food processing and packaging (Fedhusen 2000, Huss, et al., 2000, Egli et al., 2002).

1.3 The presence of *Salmonella* spp. and its relationship with indicator bacteria

The presence of *Salmonella* spp. and its relationship with indicator bacteria can be variable according to the hydrodynamic characteristics and environmental factors of the studied areas. Some studies have reported that a relation between *Salmonella* spp. and faecal bacterial-indicators was observed only rarely (Polo et al., 1998, 1999).

Because of their better survival in saline waters enterococci have been suggested to be better indicators of microbial risk in coastal and estuarine environments (Dionisio et al., 2000; Kamizoulis and Saliba, 2004; Noble et al., 2003; Polo et al., 1998; Prüss, 1998). Lemarchand and Lebaron (2003) have reported that considering the occurrence of *Salmonella* spp., besides *Giardia* sp. and *Cryptosporidium* sp. and using changes of the levels of indicator organisms, ''higher microbiological risk'' and ''lower microbiological risk'' areas can be defined. Additionally, it was reported that fecal indicators do not exactly reflect the presence of pathogens such as *Salmonella* spp. in natural waters and that pathogens and indicators may have different behaviors in the aquatic environment (Lemarchand and Lebaron 2003).

1.4 Antibiotic resistance of *Salmonella* spp. in seawater

Beta-lactam antibiotics are widely used for treatment of infections in the world. Domestic waste waters might be an important source of antibiotic-resistant *Enterobacteriaceae*. Resistances to clinically relevant antibiotics are widespread in aquatic bacteria, including potential human pathogens. Since antibiotic resistance related to domestic wastewaters is important for the ecosystem and also for human heath in the aquatic environments, the resistance frequency of some beta-lactam antibiotics to *Salmonella* spp. isolates were investigated in this study.

In this study, the presence of *Salmonella* spp. and its relationship with primary hydrographic parameters and indicator organisms of bacterial pollution (total coliform, feacal coliforms) were investigated in the various marine areas of Turkey. The results were evaluated regarding sustainable and economical usage of aquatic products, the ecosystem and human

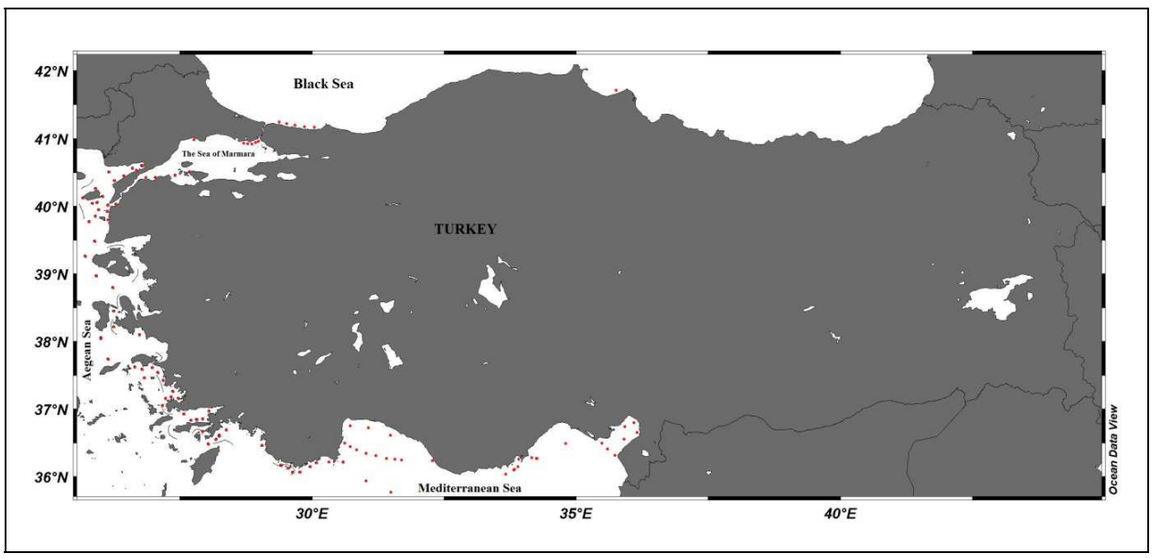

Fig. 1. Location of sampling sites in various marine areas of Turkey

health. Sea water and shellfish samples which were collected from various marine environments were investigated for occurrence of *Salmonella* spp. in different time periods throughout 1998–2010. A total of 832 samples of seawater (495), shellfish (243) and fish (94) were collected from six sites between July 1998 and August 2010.

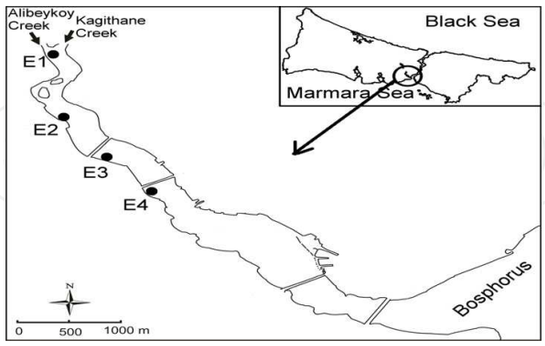

Fig. 2. One of the study areas: Golden Horn Estuary, Istanbul

2. *Salmonella* analyses

The presence of *Salmonella* spp. and indicator bacteria with respect to the areas from which they were isolated were investigated in the coastal areas of the Eastern Mediterranean, the Western Black Sea, the Golden Horn Estuary (Istanbul), the Sea of Marmara, the northern part of the Aegean Sea and also in the offshore area extending from the eastern part of Andros Island to the southern parts of Gokceada and Thasos Island, as well as the Mediterranean (Figure 1).

Indicator bacteria and *Salmonella* spp. were investigated in one hundred samples of seawater and 96 groups of *C. gallina* (striped venus*)* from six stations on the coastline of western Black Sea (Sile), Turkey. Studies were carried out on 15 days from June to December in 1998-1999 (Altuğ and Bayrak 2002).

Indicator bacteria and *Salmonella* spp. were investigated in 75 groups of sea snail (*Rapana venosa*) samples which were collected from the Florya-Ambarlı seashore of the Sea of Marmara, during the period between June 2000 and November 2001 (Altuğ and Güler 2002).

A total of 72 shellfish (*D. trunculus* /wedge-shell and *C. gallina*) were examined (36 groups *C. gallina*, 36 groups *D. trunculus*) which were taken from a site near Tekirdag on the northern coast of the Sea of Marmara, Turkey monthly between November 2005 and October 2006 (Altuğ et al., 2008).

The occurrence of *Salmonella* spp. in the total 44 samples of surface water which were collected from four different areas in the Golden Horn Estuary (Istanbul, Turkey) were tested in the period from November 2002 to December 2003.

The presence of *Salmonella* spp. in the 80 units of seawater samples, which were taken from 22 stations in the Southern part of the Sea of Marmara, was analyzed in 2006-2007 (Altuğ et. al., 2007).

The occurrence of *Salmonella* spp. in the 22 units of seawater samples from coastal areas in the Aegean Sea and 14 units of seawater samples from the Eastern Mediterranean, Turkey were investigated during the months of August in 2007 and 2008.

The occurrence of *Salmonella* spp. was investigated in the 83 units of seawater samples which were taken from various depths ranging from 0-30 cm to 500 m in the northern part of the Aegean Sea in 2006 and 2007. Seven unit samples were taken from the offshore areas

extending from the eastern part of Andros Island to the southern part of Gokceada and Thasos Island in 2007 and 2008.
The presence of *Salmonella* spp. in the 136 units of seawater samples which were taken from 68 stations in the eastern and western coastal areas of Istanbul and from around the islands in the Sea of Marmara, Turkey were investigated in 2008 and 2010.
The Sample types, the number of samples and sampling periods were summarized in Table 1.

Sample	Number of Samples	Sampling Areas (Turkey)	Sampling Period
Seawater	100	Western Black Sea	1998-1999
	44	Golden Horn Estuary (Istanbul)	2002-2003
	22	Aegean Sea (coastal areas)	2006-2008
	83	Northern Aegean Sea	2006-2007
	80	Southern part of the Sea of Marmara	2006-2007
	7	Northern Aegean Sea (0ffshore)	2007-2008
	14	Eastern Mediterranean	2007-2008
	5	Eastern Mediterranean (offshore)	2007-2008
	136	The Sea of Marmara	2008-2010
Total Seawater samples	495		
C. gallina	96 *	Western Black Sea	1998-1999
	36*	The Sea of Marmara (Tekirdağ)	2005-2006
D. trunculus	36*	The Sea of Marmara (Tekirdağ)	2005-2006
R. venosa	75*	The Sea of Marmara (Florya-Ambarlı seashore)	2000-2001
Total Shellfish Samples	243		
Fish			
Atherina boyeri	22	The Sea of Marmara (Yesilkoy-Avcılar)	1999-2000
Scorpaena porcus	24	The Sea of Marmara (Yesilkoy)	1999-2000
Spicara smaris	31	The Sea of Marmara (Yesilkoy)	1999-2000
Diplodus vulgaris	11	The Sea of Marmara (Tekirdağ)	1999-2000
Scophthalmus maeoticus	6	Black Sea (Derekoy-Samsun)	1999-2000
Total Fish Samples	94		
Total number of samples	835	Turkey	1998-2010

*A total of 6 individual samples were accepted as a sample group in the analyses

Table 1. The seawater, shellfish and fish samples which were collected from various marine environments, Turkey for bacteriological analyses in different periods.

2.1 Sampling areas

2.1.1 Western Black Sea

The Black Sea covers an area that is about one third of the area of continental Europe. The Istanbul Strait connects the Black Sea to the world's oceans. The second largest river of Europe (Danube), also large rivers such as Dnieper, Don and Dniester all flow to the Black Sea. The salinity of the Black Sea is considerably lower (about 22-26 psu) than the Mediterranean. The population in Sile, western Black Sea, the sampling area, rises to 200,000 during the months of July and August due to recreational activities, compared with 50,000 during the other months. The purpose of this study was to determine the effect of the increasing anthropological activity on the bacteriological pollution of the seawater and *C. gallina* samples.

2.1.2 The Golden Horn Estuary (Istanbul)

The Golden Horn Estuary has been heavily polluted by industrial and domestic wastes since 1950. Five million cubic meters of sludge has been removed during the last 10 years of restoration works. After the rehabilitation project, decreases in level of bacteria were reported (Altuğ and Balkıs 2009).

2.1.3 The Sea of Marmara

The Istanbul Strait connects the Sea of Marmara to the Black Sea and the Canakkale Strait to the Aegean Sea. The Sea of Marmara separates Turkey's Asian and European regions. Being an important water route between the Mediterranean and the Black Sea, the Sea of Marmara is under the pressure of heavy marine transportation. The Sea of Marmara is under the influence of various anthropological factors such as dwelling, domestic and industrial wastes. The bacteria which come from ships' ballast water are another effective factor on the composition and abundance of bacteria in the Sea of Marmara. The less saline waters of the Black Sea reach the Mediterranean via upper currents while the concentrated saline waters of the Mediterranean reach the Black Sea via the undercurrents of the Canakkale and Istanbul Straits. These interesting hydrodynamic characteristics of the Sea of Marmara offer us unique opportunities for researching bacterial composition, under different, poorly described conditions.

2.1.4 Eastern Mediterranean

Northeastern Mediterranean is known as a typical example of the world's oligotrophic seas. The salinity of the Mediterranean (38.5-38.6 psu) is considerably higher than the Black Sea. Bacterial composition of these environments have been managed by anthropological activities (Bayındırlı, 2007).

2.1.5 Aegean Sea

The pelagic zones of the northern Aegean Sea and the Sea of Marmara share some main features due to their connection through the Çanakkale Strait. However, because of the anthropological sources, bacterial pollution level of northern part of the Aegean Sea less than the Sea of Marmara (Altuğ et. al., 2007). The population rate rises during the summer season due to recreational activities, compared with the other months in the coastal areas of the Aegean Sea. This situation is inducing the level of bacterial pollution (Altuğ et. all., 2007)

2.1.6 Offshore areas

Due to the differences between coastal areas and offshore areas with respect to exposed pollution factors, the offshore areas can be accepted as reference stations for the studies which monitor bacterial contamination.

In this study, seawater samples which were taken from the offshore areas extending from the eastern part of Andros Island to the southern parts of Gokceada and Thasos Island, as well as the Mediterranean were tested for indicator bacteria and *Salmonella* spp.

2.2 Sea water sampling

The samples from close stations (western Black Sea, the Sea of Marmara, and the Golden Horn Estuary, western Black Sea) were transported daily to the Aquatic Microbial Ecology Laboratory of Faculty of Fisheries of Istanbul University.

However, because of the long distances (Northern Aegean Sea, Eastern Mediterranean) between the sampling point and the laboratory, some analyses for filtration (indicator bacteria), pre-enrichment, selective enrichment (*Salmonella* spp.) and isolation were carried out during the cruise on the Bacteriology Laboratory of the Research Vessel YUNUS-S.

The numbers of the sea water samples which were collected from various marine areas between the years 1998 and 2010 according to sampling stations were summarized in the Table 1.

2.3 Shellfish sampling

C. *gallina* samples were collected by mechanical dredge at approximately 5-10 meters depth from the western Black Sea (Sile) from June to December in 1998-1999 (Altuğ and Bayrak 2002).

R. venosa samples were collected by diving from Florya-Ambarlı seashore, (Marmara Sea, Turkey) and with the help of divers during the period between June 2000 and November 2001 (Altuğ and Güler 2002).

C. gallina and *D. trunculus* samples were harvested along 500 m of shallow (4–7-m depth) area using a mechanical dredge in a site near Tekirdag (Kumbag), on the northern coast of the Sea of Marmara, Turkey monthly between November 2005 and October 2006. The mechanical dredge used was the standard dredging equipment used in fishing; a net with mesh openings of size 6 mm is attached to the metal dredge; when the dredge is dragged by the fishing vessel, in our case for 8–10 min, those particles equal to or greater than 6-mm size are collected in the net (Altuğ et al. 2008).

All the shellfish samples for the microbiological analyses were immediately transferred to the laboratory sealed in an ice box under aseptic conditions to avoid the possibility of bacterial contamination.

2.4 *Salmonella* spp. analyses for seawater samples

Salmonella spp. analyses depend on identification with biochemical and serological tests of suspicious colonies from selective solid medium after selective enrichment and unselective prior enrichment at 37 ^{0}C in liquid medium in the seawater samples (APHA, 2000).

Then the colonies were restreaked several times to obtain pure cultures and the pure isolates of *Salmonella* spp. were identified using GN cards in the automated biochemical identification system VITEK 2 Compact 30 (Biomereux, France). The identification cards are based on established biochemical methods and newly developed substrates. There are

biochemical tests (47 tests for GN) measuring carbon source utilization, enzymatic activities, inhibition, and resistance (Pincus, 2005).

2.5 *Salmonella* analyses for shellfish samples

In the analyses, 94 groups were used; 6 individuals were accepted as a group, and a total of 10 g (25 g for *Salmonella* spp.) was taken from each of these groups to form a sample group.

In accordance with the purpose of the test, diluted homogenous solutions of samples taken from those parts that are edible, were prepared with 0.1% buffered peptone water: 25:225 for the *Salmonella* spp.

Analyses depend on identification with current biochemical and serologic tests of suspicious colonies from selective solid medium after selective enrichment for 24 h in Selenith cystine broth at a temperature of 35^0C, and unselective prior enrichment for 18–24 h at 37^0C in buffered peptone water 25:225 (w/v) (FDA, 1998). To further identify and characterize the strains that were detected and isolated, commercially available API test system (BioMerieux, France) was used. The biochemical reactions tested with API test are: production of indole; utilization of citrate; production of nitrite; fermentations of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdaline, and arabinose; production of H_2S; activities of beta-galactosidase, tryptophane desaminase, gelatinase, arginine dihydrolase, lysine decarboxylase, and ornithine decorboxylase; formation of acetoin from pyruvate and oxidase (MacDonell et al.1982, Oberhofer 1983). When there was a need to further identification, the pure isolates of suspicious colonies were identified using GN cards in the automated biochemical identification system VITEK 2 Compact 30 (Biomereux, France).

The identification cards are based on established biochemical methods and newly developed substrates. There are biochemical tests (47 tests for GN) measuring carbon source utilization, enzymatic activities, inhibition, and resistance (Pincus, 2005).

2.6 Indicator bacteria analyses

Two different methods were used for indicator bacteria analyses in various sampling periods in 1998-2010.

2.6.1 Membrane filtration method

The water samples were taken from 0-30 cm surface and from various depths ranging from 1 to 50 meters. Water samples were filtered through a 0.45 µm membrane filter with a metal vacuum filtering set (Millipore, Germany) and then the membrane filters were placed on m-Endo, m-FC and Azide-NKS for total coliform, fecal coliform and fecal streptococci. The plates were incubated for 48 h (at 37±0.1°C and 44.5±0.1°C) and the colonies on the plates were evaluated (APHA 1998; EPA 2006). Following the correction tests on suspicious colonies which grew after incubation, the average of three parallel tests was used for the numerical identification (cfu/100 mL: colony formed unit/100 mL). Brown-red colonies which grew on Azide medium were evaluated as fecal streptococci suspicious; blue colonies which grew on m-FC medium were evaluated as fecal coliform suspicious; pink-red colonies with yellow-green metallic shinyness which grew on m-Endo medium were evaluated as coliform suspicious. cytochrome oxidase test (API Strep, BioMereux) was applied to coliform suspicious colonies and oxidase negative colonies were counted. cytochrome oxidase (API Strep, BioMereux) and indole (HIMEDIA) tests were applied to fecal coliform suspicious colonies, and oxidase negative and indole positive colonies were counted. (MacFaddin 1980, APHA 2000).

2.6.2 The most probable number method

Diluted homogenous solutions of samples taken from those parts that are edible were prepared with 0.1% buffered peptone water: 10:100 for the *E. coli* total coliform and fecal coliform analyses. Sample dilutions of 10^{-1}, 10^{-2}, and 10^{-3} with buffered peptone water were transferred to three series of test tubes, each containing 10 mL of Modified Lauryl Sulphate Triptose Broth.

Analyses were done according to the three tube most probable number method (MPN) using Brilliant green bile broth (BGLB), EC broth, Eosin methylene blue agar medium, Plate count agar medium (FDA, 1998).

For characterization of coliform, Endo agar was used.

2.7 Antibiotic resistance test

The percentage of bacteria in the samples which exhibited antibiotic resistance was measured on Nutrient agar plates supplemented with Imipenem, Ampicillin, Cefotaxim, Ceftriaxon, Ceftazidim media (NCCLS 1999).

3. Occurrence of *Salmonella* spp. in the samples of seawater, shellfish and fish

3.1 Seawater

The frequency of *Salmonella* spp. according to their exposure to environmental factors in the areas from which they were isolated were shown in Table 1 in the form of summary data of the level of coliform and fecal coliform bacteria and the occurrence of *Salmonella* spp.

No *Salmonella* spp. was detected in the samples which were taken from the western Black Sea in 1998-1999.

The presence of *Salmonella* spp. in seawater from the four stations was significantly different ($p < 0.05$) in the Golden Horn Estuary, Istanbul from 2002 to 2003. Eleven of 44 seawater samples were found positive for *Salmonella* spp. The number of *Salmonella* spp. positive samples was highest in the inner part of the estuary.

The percentage distribution of the values for the ratio of fecal coliform to fecal streptococci in the surface water of the Aegean Sea and their relation with *Salmonella* spp. was also investigated. The contribution of fecal coliform bacteria to fecal streptococci (FC/FS > 0.7) showed that the sources of fecal contamination were anthropological in this area in 2006-2008. Seven of the 22 unit seawater samples were found positive for *Salmonella* spp. in the sea water samples which were taken from the coastal areas of the Aegean Sea, *Salmonella* spp. positive samples were positive correlated with the indicator bacteria count. In the five stations which have higher number of indicator bacteria than the other stations *Salmonella* spp. were found positive. The percentages of *Salmonella* spp. among the total enteric bacteria were between 25% and 37% in these stations.

Salmonella spp. was not isolated in the seawater samples which were taken from the offshore areas.

Four units of 14 seawater samples tested which were taken from coastal areas of eastern Mediterranean were found positive for *Salmonella* spp. in August 2007-2008.

Eight units of 83 seawater samples tested which were taken from 0-30 cm to 500 meters were found positive for *Salmonella* spp. in the samples of 0-30 cm, 50 meters and 100 meters in the June 2006. *Salmonella* spp. was only isolated in the summer period during the study.

Fourteen of 80 seawater samples which were taken from 30 cm to 50 meter were positive for *Salmonella* spp. in July 2006 in southern part of the Sea of Marmara. Also, three seawater samples were found *Salmonella* spp. positive in June 2007. During this study *Salmonella* spp. was isolated only in July 2006 and June 2007.

Sixty four of the 495 unit seawater samples tested was found positive for *Salmonella* spp. (13%) in the stations. Thirty three of the 64 unit *Salmonella* spp. positive samples of seawater (51.5 %) which have been recorded in the stations indicator bacteria were > 10^4 fecal coliform /100 ml.

Twenty two of 136 unit seawater samples which were taken from 0-30 cm in the Sea of Marmara were found positive for *Salmonella* spp. in the July 2009 and June 2010 period. *S. enterica* ssp. *arizonae, S. enteritidis and S. typhimurium* were the most identified isolates in the samples. *S. typhimurium* represented 64.3% of all *Salmonella* spp. strains and was identified in the seawater samples.

The frequency of *Salmonella* spp. related to fecal coliform bacteria in the seawater samples was summarized in the Table 2. Biochemical details of two of isolated *Salmonella* spp. was summarized in Table 3.

3.2 Shellfish

Eight of 243 shellfish samples analyzed were found positive for *Salmonella* spp. (3.29%). Five of eight units of *Salmonella* spp. positive samples of shellfish (83.3%) also had indicator bacteria higher than 10^4 fecal coliform /100 ml (Table 2).

Salmonella spp. was not isolated in the *C. gallina* samples which were collected from the western part of the Black Sea, Turkey in 1998 and 1999**.**

The highest levels of fecal coliform and *E. coli* within the total of 75 *R. venosa* samples analyzed were found in the samples collected during the months of August 2000 and 2001. In the samples of August 2000, *Salmonella* spp. was found positive in both samples of fecal coliform and *E. coli;* however, *Salmonella* spp. was not isolated in the other samples.

The maximum level of fecal coliform, total coliform, and *E. coli* were recorded in the *D. trunculus* and *C. gallina* samples in July, August, and September, 2006 (Altuğ et al., 2008). *Salmonella* spp. in the *D. trunculus* and *C. gallina* samples was detected only in July and August 2006.

S. typhimurium, S. enterica ssp. *arizonae* and *S. enteritidis* also was isolated among the all isolated strains from the shellfish samples.

3.3 Fish

Three *(A. boyeri, S. porcus and S.smaris)* of the 94 unit fish samples analyzed were found positive for *Salmonella* spp. in 1999. All of the *Salmonella* spp. positive samples also had indicator bacteria more than 10^4 fecal coliform /100 ml. All the isolated strains from the fish samples were *S. enterica* ssp. *arizonae*.

The overall prevalence of *Salmonella* spp. was 9.01%, with the highest occurrence in seawater (13%), shellfish (3.29 %), followed by fish (2.13%).

Thirty two of 64 *Salmonella* isolates (50%) showed resistance to Imipenem (21 isolates), Ampicillin (22 isolates), Cefotaxim (19 isolates), Ceftriaxon (11 isolates), and Ceftazidim (18 isolates) acid (9 isolates), with nine of these isolates displaying multiple resistance to four of these antibiotics.

While the highest Multiple Antibiotic Resistance (MAR) was found in the bacteria isolated in seawater which was taken from the Golden Horn Estuary, Istanbul, the bacteria isolated from northern part of the Sea of Marmara and coastal areas of Istanbul respectively followed it.

Sample Type	F. coliform	Number of *Salmonella* (+) samples	*Relation (%) between the fecal coliform level and the number of Salmonella* (+) samples
Sea Water	10-<10^2	0	0
	10^2 - <10^3	14	21.8
	10^3-<10^4	17	26.5
	>10^4	33	51.5
Number of seawater samples: 495	64 (13% of the 495 samples)		
Shellfish	10-<10^2	0	0
	10^2 - <10^3	1	12.5
	10^3-<10^4	2	25
	>10^4	5	83.3
Number of shellfish samples: 243	8 (3.3% of the 243 samples)		
Fish	10-<10^2	0	0
	10^2 - <10^3	0	0
	10^3-<10^4	3	100
	>10^4	0	0
Number of fish samples: 94	3 (2.13% of the 94 samples)		
Total number of specimens:832	75 (9.01% of the 832 samples)		

Table 2. The frequency of *Salmonella* spp. (cfu/25 ml; cfu/25 g) and fecal coliform bacteria (cfu/100 ml) in the samples

TESTS	*Salmonella* spp.	*Salmonella* spp.
APPA	-	-
ADO	-	-
PyrA	-	-
IARL	-	-
dCEL	-	-
BGAL	-	-
H2S	+	+
BNAG	-	-
AGLTp	-	-
dGLU	+	+
GGT	-	-
OFF	+	+
BGLU	-	-
dMAL	-	-
dMAN	+	+
dMNE	+	+
BXYL	-	-
BAlap	-	-
ProA	-	-
LIP	+	+
PLE	-	-
TyrA	-	-
URE	-	-
dSOR	-	-
SAC	-	-
dTAG	+	+
dTRE	+	+
CIT	-	-
MNT	-	-
5KG	-	-
ILATk	-	-
AGLU	-	-
SUCT	-	-
NAGA	-	-
AGAL	-	+
PHOS	+	+
GlyA	-	-
ODC	+	+
LDC	+	+
IHISa	-	-
CMT	-	-
BGUR	-	-
O129R	-	+

TESTS	*Salmonella* spp.	*Salmonella* spp.
GGA	-	-
IMLTa	-	-
ELLM	-	-
ILATa	-	-

APPA: *Ala-Phe-Pro-ARYLAMIDASE;* ***ADO:*** *ADONITOL;* ***PyrA:*** *L-Pyrrolydonyl-ARYLAMIDASE;* ***IARL:*** *L-ARABITOL;* ***dCEL:*** *D-CELLOBIOSE;* ***BGAL:*** *BETA-GALACTOSIDASE;* ***H2S:*** *H2S PRODUCTION;* ***BNAG:*** *BETA-ACETYL-GLUCOSAMINIDASE;* ***AGLTp:*** *Glutamyl Arylamidase pNA;* ***dGLU;*** *D-GLUCOSE;* ***GGT:*** *GAMMA-GLUTAMYL-TRANSFERASE;* ***OFF:*** *FERMENTATION/GLUCOSE;* ***BGLU:*** *BETA-GLUCOSIDASE;* ***dMAL:*** *D-MALTOSE;* ***dMAN:*** *D-MANNITOL;* ***dMNE:*** *D-MANNOSE;* ***BXYL:*** *BETA-XYLOSIDASE;* ***BAlap:*** *BETA-Alanine arylamidase pNA;* ***ProA:*** *L-Proline ARYLAMIDASE;* ***LIP:*** *LIPASE;* ***PLE:*** *PALATINOSE;* ***TyrA:*** *Tyrosine ARYLAMIDASE;* ***URE:*** *UREASE;* ***dSOR:*** *D-SORBITOL;* ***SAC:*** *SACCHAROSE/SUCROSE;* ***dTAG:*** *D-TAGATOSE;* ***dTRE:*** *D-TRHALOSE;* ***CIT:*** *CITRATE (SODIUM);* ***MNT:*** *MALONATE;* ***5KG:*** *5-KETO-D-GLUCONATE;* ***ILATk:*** *L-LACTATE alkalinisation;* ***AGLU:*** *ALPHA-GLUCOSIDASE;* ***SUCT:*** *SUCCINATE alkalinisation;* ***NAGA:*** *Beta-N-NCETYL-GALACTOSAMINIDASE;* ***AGAL:*** *ALPHA-GALACTOSIDASE;* ***PHOS:*** *PHOSPHATASE;* ***GlyA:*** *Glycine ARYLAMIDASE;* ***ODC:*** *ORNITHINE DECARBOXYLASE;* ***LDC:*** *LYSINE DECARBOXYLASE;* ***IHISa:*** *L-HISTIDINE assimilation;* ***CMT:*** *COUMARATE;* ***BGUR:*** *BETA-GLUCORONIDASE;* ***O129R:*** *O/129 RESISTANCE (comp.vibrio);* ***GGAA:*** *Glu-Gyl-Arg-ARYLAMIDASE;* ***IMLTa:*** *L-MALATE assimilation;* ***ELLM:*** *ELLMAN;* ***ILATa:*** *L-LACTATE assimilation*

Table 3. Biochemical characteristics of some isolated *Salmonella* spp which were identified using GN cards in the automated biochemical identification system VITEK 2 Compact 30 (Biomereux, France)

4. Conclusion

The frequency of *Salmonella* spp. according to their exposure to environmental factors in the areas from which they were isolated were different. For instance, higher indicator bacteria and *Salmonella* spp. abundance was found in the coastal stations compared to the offshore areas.

The *Salmonella* spp. prevalence in a total of 832 samples of seawater (495), shellfish (243), and fish (94) which were collected from six sites between 1998 and 2010 exhibited diversity according to geographical areas. The coastal areas which were under the influence of biological pollution with respect to heavy inland population displayed higher levels of *Salmonella* spp. than the offshore areas.

Enteric bacteria of sewage origin undergo a sudden osmotic shock when they enter seawater and may adapt their metabolism to the new medium by means of their osmoregulation systems. This ability of enteric bacteria aids them in gaining resistance to salt in sea environments and increases their probability of survival (Munro et al., 1989). The presence of a negative relationship between salinity concentration and the number of enteric bacteria in sea medium has been determined (Carlucci et al., 1960, APHA 1998, Bitton 2005)

In this study, the influence of salinity on the presence of *Salmonella* spp. associated with water samples was also investigated. In the Sea of Marmara it was possible to isolate *Salmonella* spp. from the under and upper stratification of various localities which possessed salinity values between 24.0 psu and 39.2 psu during the study. The bacteria levels determined in water samples taken from under the halocline layer in the Sea of Marmara were sometimes found to be higher in comparison to sea water samples taken from 0-30 cm. The higher bacteria levels found in the undercurrent were considered to be a result of deep discharge systems carrying domestic waste products. Hydrographic changeable parameters

such as seawater temperature, pH, salinity and dissolved oxygen are significant factors associated with the presence of *Salmonella* spp. In this study, seawater temperature was the only variable showing a linear positive effect on the presence of *Salmonella* in the sea, while the other parameters showed more complex nonlinear effects in the studied areas.

There are many factors such as temperature, salinity, sunlight, grazing by heterotrophic microorganisms affecting the survival of enteric bacteria in marine areas (*Sinton et al 2007:* Harm, 1980, Gameson & Gould 1985, Jagger 1985, Rozen and Belkin 2001, Sinton 2005)

Temperature also seemed to affect efficiently the abundance of indicator bacteria and *Salmonella* spp. in the study areas. *Salmonella* spp. positive samples were mostly recorded in the summer seasons and the indicator bacteria level was also higher during these periods compared to the other sampling seasons in 1998-2010. This situation is directly related to the increase of human activity, especially in coastal areas in summer seasons. However it also shows that despite the salinity stress, occurrences of indicator bacteria and *Salmonella* spp. were possible under these conditions in the seawater.

C. gallina and *D. trunculus* are two most common and abundant species in Turkish clam resources. Especially *C. gallina* is very important and valuable species, due to its great export potential, *C. gallina*, which has begun to be gathered since 1986 via mechanical dredge in Turkey, has great importance in terms of economy (Altuğ et. al., 2008).

The mean values of bacterial contamination found in the 75 *R. venosa* samples under bacteriological analysis were between 15x10 and 24x10^3 and above. It is concluded that the area is under the influence of the waste products of dwellings and naval transportation (Altuğ and Güler 2002).

Beta-lactam antibiotics are widely used for treatment of infections in the world. Domestic waste waters might be an important source of antibiotic-resistant *Enterobacteriaceae*. Resistances to clinically relevant antibiotics are widespread in aquatic bacteria, including potential human pathogens. Because antibiotic resistance related to domestic waste waters is important for the ecosystem and also for human health, the resistance frequency of *Salmonella* spp. isolates to some beta-lactam antibiotics was investigated in this study. The antibiotic derivates which were found to be resistant to bacteria were different in different regions. This situation shows that pollution input and the usage rate of antibiotics have differences related to geographic regions. Further research will help towards setting limits on the prevalence of antibiotic-resistant bacteria and supporting the effectiveness of antimicrobial agents.

It was reported that *Salmonella* spp. presence in marine waters is adequately predicted by total coliforms or fecal coliforms (Efstratiou et al. 2009). In this study, positive correlations were found between the presence of coliform bacteria (especially >10^3 cfu/100 ml) and occurrences of *Salmonella* spp. positive isolates. Efstratiou et al.(2009) reported that the *E. coli* limits set by the EU Directive for defining "good" coastal bathing water quality (500 CFU100 ml^{-1}) are much higher than the fecal coliform concentration which would best predict the absence of *Salmonella* spp.

The percentage distribution of the ratio values of Fecal Coliform to Fecal Streptococci in the surface water of the Aegean Sea and the relation of this ratio with the occurrence of *Salmonella* spp. was also investigated (Altuğ et al., 2007). The percentages of *Salmonella* spp. among total enteric bacteria were between 25% and 37%. Positive correlations were observed between the level of indicator bacteria and the presence of *Salmonella,* implying that *Salmonella* spp. occurrence is a part of anthropological pollution input in the investigated areas. The presence of isolates of *Salmonella* spp. in the marine environment is

of notable significance with respect to public health due to the potential risk of acquiring infections as a result of the consumption of contaminated aquatic products or ingestion of contaminated seawater.

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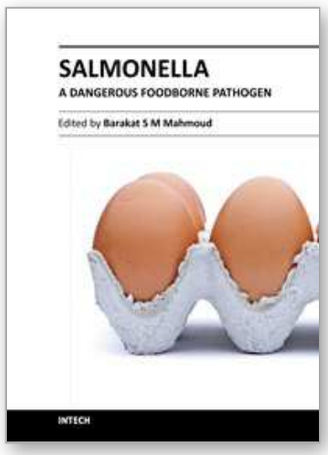

Salmonella - A Dangerous Foodborne Pathogen

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More than 2,500 serotypes of Salmonella exist. However, only some of these serotypes have been frequently associated with food-borne illnesses. Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Often, most people who suffer from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Symptoms generally occur 8 to 72 hours after ingestion of the pathogen and can last 3 to 5 days. Children, the elderly, and immunocompromised individuals are the most susceptible to salmonellosis infections. The annual economic cost due to food-borne Salmonella infections in the United States alone is estimated at $2.4 billion, with an estimated 1.4 million cases of salmonellosis and more than 500 deaths annually. This book contains nineteen chapters which cover a range of different topics, such as the role of foods in Salmonella infections, food-borne outbreaks caused by Salmonella, biofilm formation, antimicrobial drug resistance of Salmonella isolates, methods for controlling Salmonella in food, and Salmonella isolation and identification methods.

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