

## Investigation of The Presence of *Escherichia Coli* and *Salmonella* spp. in Seafood Sold in Izmir

Hatice Gündüz<sup>1\*</sup> , Fatma Öztürk<sup>1</sup> , Halime Adıgüzel<sup>1</sup> 

<sup>1</sup>Department of Fisheries and Fish Processing Technology, Faculty of Fisheries, Izmir Katip Celebi University, Izmir, Turkey

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### Corresponding Author

Hatice Gündüz

E-mail: hatice.gunduz1@ikcu.edu.tr

Tel: +90 2323293535/4259

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### Abstract

Seafood is a valuable source of animal protein that is consumed raw, partially cooked, or fully cooked in various cultures. Due to their high water and protein content, along with a near-neutral pH, they are conducive to the growth of various microorganisms. Microorganisms can be found in freshly caught seafood and can be transmitted by cross-contamination from humans during transport, gutting, sale at fish stalls or from substances added during seafood processing. *Salmonella* is not part of the natural microbiota of aquatic organisms. The presence of *Salmonella* in seafood indicates fecal contamination or cross-contamination during transport and storage. *Escherichia coli* is usually found in faecally contaminated water or food and is an indicator of poor hygiene. This study investigated the presence of *E. coli* and *Salmonella* spp. in 50 seafood products sold in Izmir. The samples subjected to analysis included trout, red mullet, sea bream, sole, frozen haddock, bogue, sea bass, seabream, saddled seabream, red porgy, stuffed mussel, striped catfish, sardine, bluefish, salema fish, salmon, mackerel, comber, anchovy, white grouper, annular sea bream, horse mackerel, oyster, picarel, squid, shrimp, chub mackerel. As a result, *E. coli* was detected in 21 of the samples analysed, while *Salmonella* spp. was detected in only 1 sample. The total coliform count was found to vary between 1.30 and 5.85 log cfu/g. These results showed that a significant amount of *E. coli* was present in seafood products and that the level of faecal contamination, and hence the potential hazard, was high. Therefore, it is necessary to increase efforts to prevent cross-contamination during the production, distribution and sale of seafood products, pay more attention to hygiene and sanitation practices, and pay special attention to personnel hygiene.

### Introduction

Seafood products are widely consumed globally due to their high nutritional value and potential

health benefits (Yang et al., 2015). Consumer preferences range from raw or minimally processed fresh products to those prepared in various forms (salted, smoked, cured, canned) and

ready-to-eat (RTE) products. In addition, seafood is an important food category in international trade and is often transported over very long distances. All these factors expose seafood to various contaminants, including those of microbiological origin (Amagliani et al., 2012).

*Salmonella* is one of the most frequently reported causes of disease and outbreaks associated with seafood around the world (Yang et al., 2015). Worldwide, there are approximately 200 million to more than 1 billion cases of *Salmonella* each year, 93 million of which result in gastroenteritis and 155,000 deaths, 85% of which are linked to food consumption (Zhou et al., 2024). The occurrence of *Salmonella* infections transmitted by fish or shellfish was documented in eight out of 160 outbreaks, representing 7.42% of the total cases (Heinitz et al., 2000).

*Salmonella* are facultative anaerobic, non-spore-forming, Gram-negative bacteria. The majority of strains are motile by means of flagella. They are mesophilic, with an optimum growth temperature of 35 to 37 °C and a growth range of 5 to 46 °C. They are sensitive to low pH (4.5 or below) and do not grow at Aw 0.94, particularly at pH 5.5 and below (Amagliani et al., 2012). The natural habitat of *Salmonella* is the gastro-intestinal tract of animals, including birds and humans (Shabarath et al., 2007).

However, *Salmonella* are not part of the normal flora of aquatic animals and the presence of these bacteria in aquatic food is a result of faecal contamination from contaminated water or cross contamination during transport or storage (Amagliani et al., 2012; Sanath Kumar et al., 2003; Yang et al., 2015). A significant corpus of epidemiological data exists concerning the presence of *Salmonella* in seafood and the diseases associated with it (Amagliani et al., 2012).

*Escherichia coli* is a Gram-negative, catalase-positive, oxidase-negative, facultative anaerobic, short rod-shaped bacterium belonging to the genus *Escherichia* of the Enterobacteriaceae family. It typically exhibits mobility with peritrichous flagella, although some strains are immobile (Değirmenci, 2017). *Escherichia coli* is a bacterium that is commonly employed for the detection of faecal contamination in water and

food due to its association with the gastrointestinal tract of warm-blooded animals. However, certain *E. coli* serovars have undergone a process of evolution, acquiring genes that confer virulence, and thus have become highly pathogenic to humans. These pathogenic *E. coli* strains can cause infection of the gastrointestinal and urinary tracts as well as the central nervous system (Prakasan et al., 2022). In developing countries, the contamination of coastal ecosystems with human and animal waste through the discharge of untreated sewage is the primary source of *Escherichia coli* in seafood (Prakasan et al., 2022).

The importance of seafood products in human nutrition is underscored by their high nutritional value. However, these products are also susceptible to a significant risk of contamination by pathogens, both existing in the product itself and subsequently transmitted to consumers. In addition to pathogens originating from the flora of the aquatic environment in which seafood is caught or cultivated, contamination can also occur from a variety of sources during the processing, storage, and transportation phases. Of particular significance are faecal contaminations. In this context, the objective of this study was to investigate the presence of *Salmonella* spp. and *Escherichia coli* in seafood products available for purchase in Izmir. The objective of this study is to contribute to the existing research on the prevalence of these bacteria in seafood products in the Izmir region. Furthermore, the data obtained aims to contribute to the existing literature on the potential risks to public health posed by aquatic products by quantifying the microbiological risks associated with their transportation, processing and storage.

## Materials and Methods

### Material

In this study, a total of 50 water products obtained from markets, bazaars and restaurants in Izmir were used for the isolation of *Escherichia coli* and *Salmonella* spp. Following the collection of samples under sterile conditions, they were transported to the laboratory under cold chain conditions (+4 °C) and subjected to microbiological analysis. The samples subjected to analysis included rainbow trout (*Oncorhynchus*

mykiss), red mullet (*Mullus barbatus*), sea bream (*Sparus aurata*), sole (*Solea solea*), frozen haddock (*Gadus merlangus euxinus*), bogue (*Boops boops*), sea bass (*Dicentrarchus labrax*), saddled seabream (*Oblada melanura*), red porgy (*Pagrus pagrus*), stuffed mussel (*Mytilus galloprovincialis*), striped catfish (*Pangasianodon hypophthalmus*), sardine (*Sardina pilchardus*), bluefish (*Pomatomus saltatrix*), salema (*Sarpa salpa*), Atlantic salmon (*Salmo salar*), mackerel (*Scomber scombrus*), comber (*Serranus cabrilla*), anchovy (*Engraulis encrasicolus*), white grouper (*Epinephelus aeneus*), annular sea bream (*Diplodus annularis*), horse mackerel (*Trachurus trachurus*), oyster (*Ostrea edulis*), picarel (*Spicara flexuosa*), squid (*Loligo vulgaris*), shrimp (*Penaeus semisulcatus*) and chub mackerel (*Scomber japonicus*).

## Method

The head and internal organs of fish were removed, before the muscle was cut into small pieces, and then completely homogenised.

### *Escherichia coli* analysis

Ten grams of the seafood sample was transferred into 90 millilitres of sterile physiological saline and homogenised. The solution was diluted to  $10^{-6}$ , ensuring adherence to the 1/10 dilution ratio. 0.1 ml of the appropriate dilutions were spread over the surface of Fluoracult Violet Red Bile Agar (FVRBA, Merck) with incubation at 37°C for 18 to 24 hours. Following incubation, red colonies surrounded by a reddish precipitate zone with a diameter of 1-2 mm were identified as coliforms. The coliform group bacteria that exhibited fluorescence under a long-wavelength UV hand lamp (Merck 1.13203) were identified as suspected *E. coli* colonies. *E. coli* ATCC (ATCC® 25922™) was used as a control during identification.

### *Salmonella* analysis

In order to ascertain the presence of *Salmonella* spp., 25 grams of the seafood samples were homogenised in 225 millilitres of buffered peptone water for pre-enrichment and incubated at 37°C for 24 hours. A quantity of 0.1 mL of the pre-enrichment culture was transferred to a test tube containing 10 mL of Rappaport-Vassiliadis Soy Broth (RVS, Merck), and incubated at 41.5 °C

for 24 hours. Following incubation, XLT4 agar was inoculated in triplicate. The inoculated Petri dishes were incubated at 37°C for 24 hours. Following incubation, colonies with black centres were deemed to be indicative of the presence of *Salmonella* spp. (Halkman, 2005).

### Biochemical identification of the bacteria

Biochemical tests were employed to identify any suspect colonies that developed following incubation. The initial step involved the evaluation of Gram reactions and microscopic morphology of the isolates.

IMViC tests (I: indole, M: methyl red, V: Voges-Proskauer, C: citrate) were employed to identify putative *E. coli* colonies (Halkman, 2005). Colonies exhibiting IMViC test results (+ + - -) were identified as *E. coli*.

In the case of colonies suspected of being *Salmonella*, an oxidase test, urea test, and a series of tests utilising Triple Sugar Iron Agar medium, lysine decarboxylase, Voges-Proskauer reaction, and indole production were conducted. The results were evaluated in accordance with the criteria set forth in Bergey's Manual of Systematic Bacteriology (LeMinor, 1984).

## Results and Discussion

As a result of the study, 50 seafood samples were tested for the presence of *E. coli* and *Salmonella*, and the total coliform count of the samples was determined (Table 1).

While *E. coli* was detected in 21 of the samples, *Salmonella* spp. was detected in only 1 sample. Total coliforms were found to vary between 1.30 and 5.85 log cfu/g in the samples. Biological agents introduced into seafood consist of bacteria, viruses and parasites that can cause serious life-threatening diseases, particularly gastroenteritis. Some of these pathogens are naturally present in the aquatic environment, while others are transmitted through contact with human or animal faeces or through sewage (Amagliani et al., 2012). The main source of transmission of *Salmonella* and other bacteria to seafood is cross-contamination during processing, transport and storage (Amagliani et al., 2012).

**Table 1.** Coliform counts, *E. coli* and *Salmonella* spp. contents of seafood samples

	Sample	Coliform (log kob/g)	<i>E. coli</i>	<i>Salmonella</i> spp.		Sample	Coliform (log Cfu/g)	<i>E. coli</i>	<i>Salmonella</i> spp.
1	Trout (Fresh-Culture)	3,58	+	-	26	Bogue 2 (Fresh)	3,04	-	-
2	Red mullet 1 (Fresh)	2,42	-	-	27	Bogue 3 (Fresh)	2,77	+	-
3	Red mullet 2 (Fresh)	5,56	-	-	28	Bogue 4 (Fresh)	2,59	-	-
4	Sea bream 1 (Fresh)	3,60	-	-	29	Sea bass 1 (Fresh)	4,07	+	-
5	Sole fish (Fresh)	2,97	+	-	30	Sea bass 2 (Fresh)	2,45	-	-
6	Frozen Haddock (Processed)	2,65	+	-	31	Seabream 3 (Fresh)	2,30	+	-
7	Comber (Fresh)	5,17	-	-	32	Saddled seabream 1 (Fresh)	3,40	-	-
8	Anchovy 1 (Fresh)	3,66	-	-	33	Saddled seabream 1 (Fresh)	3,11	-	-
9	Anchovy 2 (Fresh)	4,43	+	-	34	Red porgy (Fresh)	2,28	-	-
10	White grouper fish 1 (Fresh)	5,85	+	-	35	Stuffed mussel 1 (Processed)	2,94	-	-
11	White grouper fish 2 (Fresh)	2,60	-	-	36	Stuffed mussel 2 (Processed)	1,40	-	-
12	Annular sea bream (Fresh)	2,87	-	-	37	Striped catfish (Fresh)	3,56	+	-
13	Horse mackerel 1 (Fresh)	3,86	-	-	38	Sardine 1 (Fresh)	3,51	+	-
14	Horse mackerel 2 (Fresh)	2,64	-	-	39	Sardine 2 (Fresh)	2,47	+	-
15	Oyster (Fresh)	2,70	-	-	40	Sardine 3 (Fresh)	4,27	-	-
16	Picarel (Fresh)	2,35	+	-	41	Sardine 4	3,82	+	-
17	Sea bream 2 (Fresh)	4,32	-	-	42	Sardine 5 (Fresh)	3,12	+	-
18	Squid 1 (Fresh)	2,80	+	-	43	Sardine 6 (Fresh)	2,95	+	-
19	Shrimp (Fresh)	2,78	-	-	44	Sardine 7 (Fresh)	2,20	-	-
20	Squid 2 (Fresh)	2,45	-	-	45	Bluefish (Fresh)	3,81	-	-
21	Chub mackerel 1 (Fresh)	3,60	+	-	46	Salema porgy (Fresh)	2,56	+	-
22	Chub mackerel 2 (Fresh)	1,61	-	-	47	Salmon 1 (Fresh)	3,19	+	-
23	Chub mackerel 3 (Fresh)	2,56	-	-	48	Salmon 2 (Fresh)	3,67	-	-
24	Chub mackerel 4 (Fresh)	3,03	+	-	49	Salmon 3 (Fresh)	2,62	+	-
25	Bogue 1	2,30	-	+	50	Mackerel (Fresh)	1,30	-	-
	<b>Minimum Coliform count (log cfu/g)</b>						<b>Total number of <i>Salmonella</i> spp. samples</b>		<b>Percentage of <i>Salmonella</i> spp. samples</b>
	<b>Maximum Coliform count (log cfu/g)</b>								
	<b>1,30</b>	<b>5,85</b>	<b>3,12</b>	<b>21</b>	<b>42 %</b>	<b>1</b>	<b>2 %</b>		

In our study, *Salmonella* spp. was detected in only one (2%) of the samples analysed. Similarly, Lee (2006) reported that *Salmonella* spp. was not detected in their study of 177 seafood samples. This rate is relatively low compared to the findings in the literature. For example, Brands et al. (2005) investigated the presence of *Salmonella* spp. in oysters from the United States coast, and reported that 7.4% of the oysters analysed contained *Salmonella* spp. Similarly Shabarinath et al. (2007) reported that *Salmonella* spp. were detected in 20 of 100 seafood products analysed. In a study by Bakr et al. (2011) involving 150 samples, it was reported that *Salmonella* spp. were isolated in 10% of the samples. Furthermore, *Salmonella* spp. were isolated from shrimps, oysters and mussels. Vural and Emin Erkan (2006) identified the presence of *Salmonella* spp. in 15.69% of the 51 fish samples collected from three distinct locations along the Dicle River. Sanath Kumar et al. (2003) reported that 6 out of 20 finned fish (30%), 4 out of 20 oysters (20%) and 1 out of 20 shrimps (5%) were found to be positive for *Salmonella* spp. In a study conducted by Adesiji et al. (2014) 30 samples (6 poultry, 8 seafood and 16 clinical samples) were found to be positive for *Salmonella* spp. out of a total of 120 samples tested. Akpınar Bayizit et al. (2003) reported that 58% of the samples of frozen mussel examined were found to contain *Salmonella* spp. Atwill & Jeamsripong (2021) reported that 47% of the shrimp samples, 46% of the sea bass samples and 14-38% of the oyster samples tested positive for the presence of *Salmonella* spp. The study involved the analysis of 335 seafood products offered for sale in Thailand. Moreover, Nguyen et al. (2016), high rates of *Salmonella* contamination were detected in a variety of food products, including pork (69.7%), poultry (65.3%), beef (58.3%), shrimp (49.1%) and freshwater fish (36.6). The study involved the analysis of 409 samples. The 2% *Salmonella* spp. rate obtained in our study is relatively low in comparison to the rates reported in the existing literature (Bakr et al., 2011; Brands et al., 2005; Sanath Kumar et al., 2003; Shabarinath et al., 2007). It is hypothesised that these discrepancies are attributable to variations in hygiene standards pertaining to fishing activities, processing and storage procedures in the sampled regions.

*Salmonella* serovars are ubiquitous in the natural environment. These bacteria can gain access to the aquatic environment via wild and domestic animals, inadequate sanitation, and the improper disposal of human and animal waste (Amagliani et al., 2012). The source of *Salmonella* spp. in fish and other aquatic products has been identified as contamination of the water source or contamination occurring during storage and processing (Amagliani et al., 2012). *Salmonella* spp. are among the most common food-associated pathogens and are responsible for a significant proportion of deaths resulting from foodborne diseases (Brands et al., 2005).

The analysis of the samples revealed the presence of *Salmonella* spp. only in fresh bogue fish. The low prevalence of *Salmonella* spp. in seafood products sourced from the Izmir region suggests that the processing and storage of seafood products in this region adhere to more rigorous hygienic standards. In a study conducted in Thailand, Atwill & Jeamsripong (2021) detected a prevalence of 47% for *Salmonella* spp. in shrimps and 46% in sea bass. It is presumed that this difference between the studies is a consequence of more rigorous hygiene protocols and processing procedures.

The results of our study indicated that 21 (42%) of the samples analysed were positive for *E. coli*. Moreover Akpınar Bayizit et al. (2003) reported that *E. coli* was not detected in frozen mussel samples. This finding is significantly at odds with the results of several studies previously reported in the literature. Prakasan et al. (2022) reported that *E. coli* was detected in 75 (96.2%) of the 78 seafood samples included in their study on finned fish and shellfish. Similarly, Atwill & Jeamsripong (2021) reported that 335 seafood products offered for sale in Thailand were positive for faecal coliform, with 85% of these samples also containing *E. coli*. The rates of *E. coli* detected in these studies are considerably higher than those observed in the study conducted by our research group. It is hypothesised that these discrepancies are attributable to variations in geographical factors, hygiene standards and practices employed in the catching, processing and storage of seafood products.

The study conducted by Matyar et al. (2008) in Iskenderun Bay revealed the absence of *E. coli* in 97 shrimp samples. This finding suggests that the environmental conditions in Iskenderun Bay may be more favourable than those observed in the Izmir region. Furthermore, the study by Matyar et al. (2008) revealed the presence of *E. coli* in water and sediment samples collected from the same areas where fishing took place. This finding indicates that seafood products may be susceptible to contamination from polluted water sources, underscoring the importance of water quality during the fishing process. The presence of *E. coli* in seafood may serve as an indicator of faecal contamination and inadequate processing methods (Chakravarty et al., 2015). Furthermore, contamination may be associated with additional factors, including water quality, fishing methodology, storage conditions, and processing procedures. The *E. coli* rate in the Izmir region may indicate that the risk of faecal contamination is low; however, it also suggests the necessity for improvements in regional hygiene standards. These findings are corroborated by the supposition that fishing operations in the Izmir region are conducted in accordance with more rigorous regulatory standards. However, in comparison to the elevated *E. coli* levels documented by Prakasan et al. (2022) and Atwill & Jeamsripong (2021), the diminished *E. coli* concentrations identified in the Izmir region may be attributable to regional disparities in environmental conditions or hygiene standards in seafood processing. Nevertheless, further comprehensive research across diverse seasons and regions is essential to ascertain the underlying reasons for the observed discrepancy in *E. coli* rates between the Izmir region and other areas.

Consequently, the prevalence of *Escherichia coli* in seafood originating from the Izmir region was found to be lower than that reported in numerous studies published in the scientific literature. This represents a novel contribution to the field of seafood safety in Turkey, underscoring the beneficial influence of local hygiene standards on food safety.

In the course of our study, *E. coli* was identified in 42% of the 50 seafood products subjected to analysis, while *Salmonella* spp. was detected in

only 2%. The discrepancy in the detection rates of these two bacteria may suggest that high faecal coliforms do not necessarily indicate the presence of *Salmonella*. A comparable result was observed in the literature by Brands et al. (2005). The researchers observed no significant correlation between faecal coliform counts in oysters and the isolation of *Salmonella*. Furthermore, they posited that high faecal contamination does not invariably increase the risk of *Salmonella* infection, and that different bacterial contamination sources may be present. The low prevalence of *Salmonella* spp. despite the high *E. coli* rates observed in the present study suggests the existence of diverse faecal contamination sources and differences in the transmission routes of these pathogens. This result indicates that the responses of *E. coli* and *Salmonella* to environmental factors and hygiene conditions may be disparate.

A multitude of factors, including insufficient access to clean water, inadequate hygiene practices, and the absence of effective food safety measures, have been identified as key contributors to the increasing incidence of foodborne salmonellosis cases (Shabarinath et al., 2007). The most common factors contributing to salmonellosis outbreaks are inadequate cooking, improper storage, cross-contamination and the use of raw ingredients in the preparation of seafood (Amagliani et al., 2012). Although the prevalence of *Salmonella* spp. was low in our study, it should be noted that this may not be the case in unhygienic processing conditions. Given the susceptibility of seafood products to bacterial contamination and the potential for such contamination to pose health risks to consumers when appropriate conditions are not created Bakr et al. (2011), It is therefore of great importance to minimise microbial risks in order to ensure the safety of seafood products. The elevated detection of *E. coli*, an indicator of faecal contamination in seafood, highlights the potential health risks associated with its presence. The mere presence of non-pathogenic bacteria such as *E. coli* is of critical importance to public health, as it may indicate the presence of other enteric pathogens of faecal origin (Costa, 2013).

In order to safeguard the microbiological quality and safety of seafood products, it is essential to implement the following basic measures.

1. Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) are fundamental to ensuring the quality and safety of seafood products. It is imperative that rigorous adherence to hygiene standards be observed throughout the entirety of the processing procedures.

2. Sanitation Standard Operating Procedures (SSOPs) and Hazard Analysis at Critical Control Points (HACCP) are essential for ensuring the safety and quality of seafood products. These systems must guarantee that microbiological safety is monitored at all stages of the production process, from the initial stages of seafood production until the final product reaches the consumer (Amagliani et al., 2012).

3. Post-harvest care is a crucial aspect of the seafood production process. It is of paramount importance that seafood is stored and processed under appropriate conditions following its capture in order to minimise the risks of contamination.

4. It is recommended that raw or undercooked seafood be avoided. It is important to reiterate to consumers that the consumption of raw or undercooked seafood carries an inherent health risk (Costa, 2013).

The implementation of enhanced regional hygiene standards and the establishment of a continuous monitoring programme for microbiological quality will serve to enhance the safety of seafood products for human consumption.

## Conclusions

The present study aimed to determine the presence of *Salmonella* spp. and *E. coli* in seafood products available for purchase in Izmir. The findings indicate that these bacteria pose a significant microbiological risk to public health. The particularly elevated *E. coli* levels suggest that the sampled seafood products are susceptible to faecal contamination. While the low prevalence of *Salmonella* spp. in the Izmir region indicates that regional hygiene standards may be effective, the value of the results is limited due to the limited sample size and data collection from a single region. Further studies conducted across different seasons and geographical regions will help generate more consistent and generalized findings on seafood safety.

## Ethical approval

The author declares that this study complies with research and publication ethics.

## Informed consent

Not available

## Conflicts of interest

There is no conflict of interests for publishing of this study.

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## Author contribution

All authors contributed equally to the writing of the manuscript, read and approved the final version.

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