# Microbial threats in raw food products: prevalence of bacterial pathogens

## *Ameaças microbianas em produtos alimentícios crus: prevalência de patógenos bacterianos*

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### Abstract

Food safety has emerged as a major global public health concern due to microbiological risks associated with raw food products. This study aimed to determine the prevalence of bacterial pathogens in raw beef, raw chicken, fish, leafy greens (lettuce, parsley, spinach), and raw milk samples collected in Kocaeli, Türkiye, between May and October 2024. A total of 220 samples were analyzed using standardized microbiological methods for E*scherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Shigella* spp. The colony counts were determined and expressed as CFU/g or CFU/mL according to ISO protocols. *E. coli* was detected in 59.54% of the samples analyzed, *S. aureus* in 31.81%, *Salmonella* spp. in 10%, and *L. monocytogenes* in 0.9%. The detected microbial counts, particularly for *E. coli* and *S. aureus*, exceeded the limits established by the Turkish Food Codex and EU regulations for raw food products, indicating potential health risks. The elevated microbial counts may be explained by hygiene deficiencies during production, handling, and storage, including inadequate sanitation, contaminated equipment, and environmental exposure. *E. coli* was the most frequently detected bacterium, particularly in raw milk (80%) and leafy greens (75%). *Salmonella* spp. was mainly found in raw chicken (17.5%) and fish meat (11.9%), while *S. aureus* was most prevalent in raw milk (53.33%) and fish meat (47.61%) samples. *L. monocytogenes* was detected at low levels only in raw beef (2.04%) and raw chicken (2.5%), while *Shigella* spp. was not detected in any of the samples. The results suggest that hygiene deficiencies may be among the potential factors contributing to contamination, along with other possible contamination sources throughout the production and handling chain.

**Keywords:** Foodborne pathogens. Raw food safety. Microbiological contamination. Public health risk. Hygiene.

### *Resumo*

*A segurança alimentar tornou-se uma grande preocupação global de saúde pública devido aos riscos microbiológicos associados aos produtos alimentícios não processados. Este estudo teve como objetivo determinar a prevalência de*

*patógenos bacterianos em amostras de carne bovina crua, frango cru, peixe, hortaliças folhosas (alface, salsa, espinafre) e leite cru coletadas em Kocaeli, Turquia, entre maio e outubro de 2024. Um total de 220 amostras foram analisadas utilizando métodos microbiológicos padronizados para* Escherichia coli, *S*taphylococcus aureus, Salmonella *spp.,* Listeria monocytogenes *e* Shigella *spp. A contagem de colônias foi determinada e expressa em UFC/g ou UFC/ml, de acordo com os protocolos ISO.* E. coli *foi detectada em 59,54% das amostras analisadas,* S. aureus *em 31,81%,* Salmonella *spp. em 10% e* L. monocytogenes *em 0,9%. As contagens microbianas detectadas, particularmente para* E. coli *e* S. aureus*, excederam os limites estabelecidos pelo Codex Alimentar Turco e pelas regulamentações da UE para produtos alimentícios crus, indicando potenciais riscos à saúde. A elevada contagem microbiana pode ser explicada por deficiências de higiene durante a produção, manuseio e armazenamento, incluindo saneamento inadequado, equipamentos contaminados e exposição ambiental.* E. coli *foi a bactéria detectada com maior frequência, particularmente no leite cru (80%) e em vegetais folhosos (75%).* Salmonella *spp. foi encontrada principalmente em frango cru (17,5%) e carne de peixe (11,9%), enquanto* S. aureus *foi mais prevalente em amostras de leite cru (53,33%) e carne de peixe (47,61%).* L. monocytogenes *foi detectada em baixos níveis apenas em carne bovina crua (2,04%) e frango cru (2,5%), enquanto* Shigella *spp. não foi detectada em nenhuma das amostras. Os resultados sugerem que as deficiências de higiene podem estar entre os fato-res potenciais que contribuem para a contaminação, juntamente a outras possíveis fontes de contaminação ao longo da cadeia de produção e manuseio.*

***Palavras-chave:*** *Patógenos transmitidos por alimentos. Segurança de alimentos crus. Contaminação microbiológica. Risco à saúde pública. Higiene.*

## Introduction

While microorganisms play important roles in food production through fermentation processes, probiotic supplementation, and biotechnological applications, the control of pathogenic microorganisms is a critical factor in food safety, hygiene practices, and microbial risk assessment. In addition, microorganisms can directly affect shelf life and consumer health by contributing to food spoilage through lipid oxidation, protein hydrolysis, and organoleptic changes (Saucier, 2016).

Pathogens or harmful microorganisms present in food products cause significant public health problems and are among the leading causes of illness and death (Abebe et al., 2020). Bacterial contamination occurs through direct or indirect contact with objects contaminated with feces. This contamination can spread via food, water, fingernails, and hands, and is particularly transmissible through the fecal-oral route in cases of poor hygiene. Such conditions enable infected individuals to transmit microorganisms to others by touching surfaces or through direct physical contact, facilitating the rapid spread of diseases within communities (Marriott et al., 2018). According to the World Health Organization (WHO), approximately 600 million cases each year are attributed to foodborne illnesses, leading to fatal outcomes particularly among vulnerable population groups (Lee and Yoon, 2021).

Raw foods are widely preferred around the world due to their nutritional properties and minimal processing requirements. However, the microbiological risks associated with the consumption of these foods have become a significant public health concern. In particular, products such as raw beef, chicken, fish, leafy greens (lettuce, parsley, spinach), and raw milk provide a favorable environment for the transmission of pathogens (EFSA, 2023). Among the main microorganisms causing infections are pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus*. These bacteria pose a serious threat to public health by causing contamination at various stages of the food chain (Gourama, 2020).

*E. coli* is a facultative anaerobic, Gram-negative, rod-shaped bacterium that is naturally present in the intestinal flora of humans and animals. However, certain pathogenic serotypes can cause foodborne infections. Contaminated water, undercooked meat, and greens grown under unhygienic conditions are among the main sources of *E. coli* transmission (Suleman et al., 2022).

*Salmonella* spp. is a rod-shaped, Gram-negative, facultative anaerobic, motile bacterial genus that typically does not ferment lactose and belongs to the Enterobacteriaceae family. It is recognized as one of

the most common pathogens in foodborne infections worldwide. Animal products such as poultry meat and eggs are among the primary sources of contamination. Salmonella infections are generally characterized by gastroenteritis; however, in immunocompromised individuals, severe cases may lead to sepsis and fatal complications (Popa and Papa, 2021).

*L. monocytogenes* is a short rod-shaped, Gram-positive, facultative anaerobic, motile, and catalase-positive bacterium belonging to the Listeriaceae family. Due to its psychrotrophic nature, this bacterium has the ability to grow at low temperatures. It is commonly found in raw milk and dairy products. Listeriosis is a significant foodborne infection that can lead to severe complications such as fetal loss in pregnant women and meningitis in newborns, particularly affecting individuals with weakened immune systems (Ribeiro et al., 2023).

*S. aureus* is a Gram-positive, catalase- and coagulase-positive, facultative anaerobic bacterium that appears in spherical or grape-like clusters and belongs to the Staphylococcaceae family. Due to its ability to produce heat-resistant enterotoxins, it is one of the main causes of foodborne intoxications. It commonly contaminates food in environments with poor hygiene and is frequently found in foods handled by hand. The toxins it produces pose a significant public health risk by causing foodborne illnesses characterized by rapid onset of gastrointestinal symptoms such as nausea, vomiting, and diarrhea (Pal et al., 2023).

*Shigella* spp. is a short rod-shaped, Gram-negative, non-motile, facultative anaerobic bacterial genus that belongs to the Enterobacteriaceae family. It typically does not ferment lactose and can cause disease with a very low infectious dose. Due to its low infectious dose, this highly contagious pathogen is frequently detected in contaminated water and in greens grown under poor hygienic conditions. Shigellosis, characterized by severe diarrhea and bloody stools, represents a significant public health concern, particularly in developing countries (Bennish and Ahmed, 2020).

In the assessment of microbiological risks, the Turkish Food Codex Regulation on Microbiological Criteria serves as a fundamental reference. This regulation plays a crucial role in ensuring food safety by establishing limit values for microorganisms permitted in food products. For instance, the presence of pathogens such as *Salmonella* spp. and *L. monocytogenes* in certain food categories is considered unacceptable from a public health perspective, whereas microorganisms such as *E. coli* and *S. aureus* are allowed up to specific levels. However, exceeding these limits poses a serious threat to food safety (TGK, 2025).

In designing prevalence studies, appropriate sample size determination is a critical factor. For this study, the sample size of 220 was determined using epidemiological sample size estimation methods, considering an expected prevalence of 10–20%, 95% confidence level, and 5% margin of error. This approach ensured that the collected samples provide statistically reliable estimates for pathogen prevalence across multiple raw food groups.

This study aims to assess the prevalence of bacterial pathogens in raw foods and to provide strategic recommendations to ensure food safety, based on standardized microbiological evaluations. Although many studies have focused on individual food types or specific pathogens, there is limited data comparing multiple food groups within a localized surveillance context using internationally validated standards. Therefore, we hypothesize that raw food products in Kocaeli, Türkiye, may exhibit considerable contamination, potentially influenced by multiple factors such as possible hygiene-related issues, processing conditions, environmental contamination, or failures occurring at different stages of the food chain.

## Material and methods

Between May and October 2024, a total of 220 raw food samples were collected from butcher shops and marketplaces in Kocaeli Province, Türkiye. These included 49 raw beef, 40 raw chicken, 42 fish meat, 44 leafy greens (15 parsley, 15 lettuce, 14 spinach), and 45 raw milk samples taken directly from boilers located in livestock production areas. Sampling followed a stratified random strategy across four major regions of the province, proportionally representing the availability and consumption frequency of each food group. Samples were transported under cold chain conditions (4 to 8 °C) and processed on the same day.

The same set of 220 samples was previously analyzed *E. coli* (STEC) serotypes (Soycan et al., 2025), but

that work exclusively targeted stx, eae, and specific serogroup genes using Real-Time PCR. In contrast, the present study investigates different bacterial groups (*E. coli*, *Salmonella* spp., *L. monocytogenes*, *S. aureus*, *Shigella* spp.) using classical culture-based ISO methods; therefore, the datasets and analytical endpoints are independent.

All microbiological procedures followed internationally validated standards (ISO methods harmonized with Codex Alimentarius and the Turkish Food Codex), ensuring reliability, reproducibility, and regulatory compliance.

Although the included food groups differ in production workflows and in their respective Good Manufacturing Practices/Hazard Analysis Critical Control Points requirements, they were intentionally evaluated together to generate surveillance-based, cross-sectional epidemiological data under the same regional and temporal conditions. This multi-matrix design enables the identification of pathogen distribution patterns across raw foods commonly consumed in Kocaeli and supports risk-based monitoring without attempting to compare technological processing differences among food types.

**Isolation and identification of *E. coli***

A 10 g/mL portion of each sample was weighed into sterile bags, and 90 mL of Buffer Peptone Water (BPW) (NCM0015A, Neogen, UK) was added. The mixture was homogenized in a stomacher for 30–40 seconds. From the prepared dilution, 1 mL was transferred to an empty sterile Petri dish, and approximately 15 mL of Tryptone Bile Glucuronide Agar (NCM1001A, Neogen, UK), preheated to 44–47 °C, was poured onto it. The plate was gently swirled to mix and then incubated at 44 °C for 18–24 hours. After incubation, blue or blue-green colonies were considered presumptive *E. coli* colonies and were subjected to confirmatory IMViC tests prior to colony counting (ISO 16649-2, 2001).

Only fish samples were analyzed for *E. coli* colony counts using the Most Probable Number (MPN) method. A 10 g portion of the sample was weighed into sterile bags, and 90 mL of BPW was added. The mixture was homogenized in a stomacher for 30–40 seconds, resulting in a $10^{-1}$ dilution. A total of nine tubes were prepared in a 3-3-3 format using Mineral Modified Glutamate Medium (NCM0186K1, Neogen, UK); the first three tubes were prepared with double strength medium, and the remaining six with single strength. Into the first three double-strength tubes, 10 mL of the $10^{-1}$ dilution was inoculated. The next three single-strength tubes received 1 mL of the $10^{-2}$ dilution, and the last three tubes were inoculated with 1 mL of the $10^{-3}$ dilution. All tubes were incubated at 37°C for 24 ± 2 hours. After incubation, tubes showing acid production and yellow coloration were considered presumptive positive. From each suspected tube, a loopful was streaked onto Tryptone Bile Glucuronide Agar and incubated at 44 °C for 20–24 hours. At the end of incubation, any tube associated with blue or blue-green colonies on the Petri dish was considered *E. coli* positive, and the result was evaluated using the MPN table (ISO16649-3, 2015).

**Isolation and identification of *Salmonella* spp.**

For pre-enrichment, 25 g/mL of the sample was weighed into sterile bags, and 225 mL of BPW was added. The mixture was homogenized in a stomacher for 30–40 seconds and incubated at 37 °C for 18–24 hours. For selective enrichment, 0.1 mL of the pre-enrichment homogenate was transferred into Rappaport-Vassiliadis Broth (NCM0136A, Neogen, UK) and incubated at 42 ± 1°C for 18–24 hours. After incubation, a loopful from the selective enrichment was streaked onto Xylose Lysine Deoxycholate (XLD) Agar (NCM0021A, Neogen, UK), a selective differential medium, and incubated again at 37 °C for 18–24 hours. Colonies that grew with a black center and were positive for lactose and lysine decarboxylase tests were considered presumptive *Salmonella* spp.

Five presumptive *Salmonella* colonies were selected and subjected to biochemical tests including Gram staining, oxidase, catalase, Triple Sugar Iron Agar (TSI), Lysine Iron Agar (LIA), and API 20E (bio Mérieux/France). The results were confirmed based on these tests (ISO 6579–1, 2017). Additionally, colonies that tested positive were serotyped using O, H, and Vi antigen sera to identify and classify *Salmonella* spp. (ISO 6579–1, 2017).

All steps of *Salmonella* detection were performed strictly according to ISO 6579-1 (2017), including non-selective pre-enrichment in BPW, selective enrichment in Rappaport-Vassiliadis Broth, isolation on XLD agar, biochemical confirmation, and serotyping (ISO 6579–1, 2017).

### Isolation and identification of *L. monocytogenes*

For pre-enrichment, 25 g/mL of the sample was weighed into sterile bags, and 225 mL of Half Fraser Broth (NCM001A, Neogen, UK) was added. The mixture was homogenized in a stomacher for 30–40 seconds and incubated at 30 ± 1 °C for 24 hours. After incubation, 0.1 mL of the pre-enrichment homogenate was transferred into tubes containing 10 mL of Fraser Broth (NCM0050A, Neogen, UK) for selective enrichment and incubated at 37 °C for 24–48 hours. Following this, a loopful of culture was streaked onto Harlequin Listeria Chromogenic Agar (Ottaviani & Agosti-ALOA; NCM1004A, Neogen, UK) and incubated again at 37 ± 1°C for 24–48 hours. Colonies appearing blue-green with an opaque halo on ALOA were considered presumptive *L. monocytogenes*. Confirmation of the isolates was performed using motility and CAMP tests, as well as the commercial Microgen Listeria ID kit (Microgen Bioproducts Ltd, UK), in accordance with ISO 11290-1 (2017).

### Isolation and identification of *S. aureus*

A 10 g/mL portion of the sample was weighed into sterile bags. BPW was added, and the mixture was homogenized in a stomacher for 30–40 seconds. From the initial dilution, a total of 1 mL (0.3–0.3–0.4 mL) was inoculated onto previously prepared Petri dishes containing Baird Parker Agar (NCM0200A, Neogen, UK) using the spread plate method, and incubated at 37 °C for 24–48 hours. After incubation, typical colonies were identified as black or gray, shiny, and convex. Atypical colonies were confirmed using both the Microgen Staph ID (Microgen, Surrey, UK) and the Tube Coagulase Test. Colonies that tested positive in both tests whether typical or atypical were counted, and the results were calculated accordingly (ISO 6888-1, 2021).

### Isolation and identification of *Shigella* spp.

For pre-enrichment, 25 g/mL of the sample was weighed into sterile bags, and 225 mL of BPW was added. The mixture was homogenized in a stomacher for 30–40 seconds and incubated at 37 °C for 18–24 hours. For selective enrichment, 1 mL of the pre-enrichment homogenate was transferred into Shigella Broth (M1326, Himedia, India) and incubated at 42 ± 1 °C for 18–24 hours. Following incubation, a loopful from the selective enrichment was streaked onto XLD Agar and incubated again at 37 °C for 18–24 hours. Colonies that did not produce hydrogen sulfide and appeared colorless or changed from pale yellow to orange were considered presumptive *Shigella* spp.

Five presumptive *Shigella* colonies were selected and subjected to biochemical tests including Gram staining, oxidase, catalase, TSI, LIA, and API 20E. The results were confirmed based on these tests (ISO 21567, 2004).

### Control

*E. coli* NCTC 12923, *S. aureus* NCTC 10788, *S. Typhimurium* ATCC 14028 and NCTC 12923, *L. monocytogenes* NCTC 11994, and *S. flexneri* NCTC 4839 strains were used as positive controls.

### Statistical analysis

Chi-square test and proportion comparison were performed to evaluate the differences in positivity rates among isolates obtained from different products. Data analysis was conducted using SPSS 24.0 (SPSS Inc., Chicago, USA), and $p < 0.05$ was considered statistically significant.

### Ethical approval

This study was conducted in accordance with ethical guidelines and was approved by the Non-Interventional Clinical Research Ethics Committee of Kocaeli University (approval number: GOKAEK-2024/07.25).

## Results

*E. coli* was detected in 55.10% (27/49), *S. aureus* in 16.32% (8/49), *Salmonella* spp. in 8.16% (4/49), and *L. monocytogenes* in 2.04% (1/49) of the raw beef samples. In raw chicken samples, *E. coli* was found in 45% (18/40), *S. aureus* in 17.5% (7/40), *Salmonella* spp. in 17.5% (7/40), and *L. monocytogenes* in 2.5% (1/40). In fish meat samples, *E. coli* was detected in 40.47% (17/42), *S. aureus* in 47.61% (20/42), and *Salmonella* spp. in 11.9% (5/42).

Among leafy green samples, *E. coli* was present in 75% (33/44), *S. aureus* in 31.81% (14/44), and *Salmonella* spp. in 6.81% (3/ 44). In raw milk samples, *E. coli* was detected in 80% (36/45), *S. aureus* in 53.33% (21/45), and *Salmonella* spp. in 6.66% (3/45). *L. monocytogenes* was not de-tected in fish, leafy green, or raw milk samples, and *Shigella* spp. was not detected in any of the analyzed samples (Table 1 and Figure 1) ($p > 0.05$).

A total of three *Salmonella* spp. were detected in leafy green samples, with one found in parsley and two in spinach. *E. coli* was detected in 33 samples overall, of which nine were from lettuce (60%), 11 from parsley (73.33%), and 13 from spinach (92.85%). *S. aureus* was identified in 14 samples in total, including three from lettuce (20%), seven from parsley (46.66%), and four from spinach (28.57%) ($p > 0.05$) (Table 2).

*Salmonella* spp. was among the most prevalent pathogens in raw chicken meat, while *E. coli* showed the highest prevalence in raw milk (Tables 3 and 4). The highest contamination of *S. aureus* was detected in raw milk and fish meat samples (Table 4).

Statistical analysis revealed that *E. coli* contamination was significantly higher in fish meat, leafy greens, and raw milk samples ($p < 0.05$). This finding suggests that fecal contamination may pose a notable issue in these food groups and may indicate the need for improved hygiene practices throughout the production-to-consumption chain. Particularly, the high levels of *E. coli* detected in raw milk samples suggest possible inadequacies in milking hygiene and a potential risk of cross-contamination during milk processing.

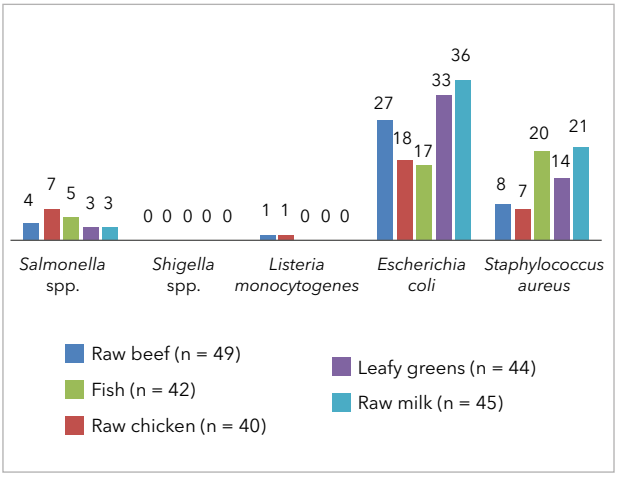


**Figure 1** - Bar chart showing the number of bacteria detected in raw food samples.

**Table 1** - Distribution of pathogenic bacteria detected in raw food products

| Sampels (n) | Number of positive samples (%) | | | | |
|---|---|---|---|---|---|
| | *Salmonella* spp. | *Shigella* spp. | *Listeria monocytogenes* | *Escherichia coli* | *Staphylococcus aureus* |
| Raw beef (n = 49) | 4 (8.16) | - | 1 (2.04) | 27 (55.10) | 8 (16.32) |
| Raw chicken (n = 40) | 7 (17.50) | - | 1 (2.50) | 18 (45.00) | 7 (17.50) |
| Fish (n = 42) | 5 (11.90) | - | - | 17 (40.47) | 20 (47.61) |
| Leafy greens (n = 44) | 3 (6.81) | - | - | 33 (75.00) | 14 (31.81) |
| Raw milk (n = 45) | 3 (6.66) | - | - | 36 (80.00) | 21 (53.33) |
| Total (n = 220) | 22 (10.00) | - | 2 (0.90) | 131 (59.54) | 70 (31.81) |

**Table 2** - Distribution of pathogenic bacteria in leafy greens

| Sampels (n) | Number of positive samples (%) | | | | |
|---|---|---|---|---|---|
| | *Salmonella* spp. | *Shigella* spp. | *Listeria monocytogenes* | *Escherichia coli* | *Staphylococcus aureus* |
| Lettuce (n = 15) | - | - | - | 9 (60.00) | 3 (20.00) |
| Parsley (n = 15) | 1 (6.66) | - | - | 11 (73.33) | 7 (46.66) |
| Spinach (n = 14) | 2 (14.28) | - | - | 13 (92.85) | 4 (28.57) |
| Total (n = 44) | 3 (6.81) | - | - | 33 (75.00) | 14 (31.81) |

**Table 3** - Statistical comparison of *Salmonella* spp., *Shigella* spp. and *Listeria monocytogenes* across different raw food categories

| Samples (n = 220) | ***Salmonella* spp.** n (%) | | | ***Shigella* spp.** n (%) | | | ***L. monocytogenes*** n (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | P (n = 22) | N (n = 198) | p-value | P (n = 0) | N (n = 220) | p-value | P (n = 2) | N (n = 218) | p-value |
| RB (n = 49) | 4 (18.18) | 45 (22.72) | > 0.050 | - | 49 (100) | > 0.050 | 1 (2.04) | 48 (97.95) | > 0.050 |
| Ot (n = 171) | 18 (81.81) | 153 (77.27) | | - | 171 (100) | | 1 (0.58) | 170 (99.41) | |
| RC (n = 40) | 7 (17.50) | 33 (82.50) | > 0.050 | - | 40 (100) | > 0.050 | 1 (2.50) | 39 (97.50) | > 0.050 |
| Ot (n = 180) | 15 (8.33) | 165 (91.66) | | - | 180 (100) | | 1 (0.55) | 179 (99.44) | |
| F (n = 42) | 5 (11.90) | 37 (88.09) | > 0.050 | - | 42 (100) | > 0.050 | - | 42 (100) | > 0.050 |
| Ot (n = 178) | 17 (9.55) | 161 (90.44) | | - | 178 (100) | | - | 178 (100) | |
| GL (n = 44) | 3 (6.81) | 41 (93.18) | > 0.050 | - | 44 (100) | > 0.050 | - | 44 (100) | > 0.050 |
| Ot (n = 176) | 19 (10.79) | 157 (89.20) | | - | 176 (100) | | - | 176 (100) | |
| RM (n = 45) | 3 (6.66) | 42 (93.33) | > 0.050 | - | 45 (100) | > 0.050 | - | 45 (100) | > 0.050 |
| Ot (n = 175) | 19 (10.85) | 156 (89.14) | | - | 175 (100) | | - | 175 (100) | |

Note: P = positive; N = negative; Ot = other; RB = raw beef; RC = raw chicken; F = fish; GL = green leafy; RM = raw milk.

**Table 4** - Statistical comparison of *Escherichia coli* and *Staphylococcus aureus* across different raw food categories

| Samples (n = 220) | ***Escherichia coli*** n (%) | | | ***Staphylococcus aureus*** n (%) | | |
|---|---|---|---|---|---|---|
| | Positive (n = 131) | Negative (n = 89) | p-value | Positive (n = 70) | Negative (n = 150) | p-value |
| Raw beef (n = 49) | 27 (55.10) | 22 (44.89) | > 0.050 | 8 (16.32) | 41 (83.67) | **0.014** |
| Other (n = 171) | 104 (60.81) | 67(39.18) | | 62 (36.25) | 109 (63.74) | |
| Raw chicken (n = 40) | 18 (45.00) | 22 (55.00) | > 0.050 | 7 (17.50) | 33 (82.50) | **0.049** |
| Other (n = 180) | 113 (62.77) | 67 (37.22) | | 63 (35.00) | 117 (65.00) | |
| Fish (n = 42) | 17 (40.47) | 25 (59.52) | **0.009** | 20 (47.61) | 22 (52.38) | **0.023** |
| Other (n = 178) | 114 (64.04) | 64(35.95) | | 50 (28.08) | 128 (71.91) | |
| Leafy greens (n = 44) | 33 (75.00) | 11 (25.00) | **0.0031** | 14 (31.81) | 30 (68.18) | > 0.050 |
| Other (n = 176) | 98 (55.68) | 78 (44.31) | | 56 (31.81) | 120 (68.18) | |
| Raw milk (n = 45) | 36 (80.00) | 9 (20.00) | **0.003** | 21 (46.66) | 24 (53.33) | **0.027** |
| Other (n = 175) | 95 (54.28) | 80 (45.71) | | 49 (28.00) | 126 (72.00) | |

Note: Bold values indicate statistically significant differences ($p < 0.05$).

The significant *E. coli* contamination found in leafy green samples suggests that irrigation water used in agricultural production may be exposed to fecal contamination. The significant *E. coli* contamination detected in fish meat samples indicates that water sources may carry fecal pollution and highlights the need to strengthen hygiene standards in fish processing facilities. Additionally, *S. aureus* contamination was found to be statistically significant in raw beef, raw chicken, fish meat, and raw milk samples ($p < 0.05$).

These results may indicate possible hygiene-related issues in animal food processing, along with other potential contributing factors such as improper handling, processing conditions, or cross-contamination risks. They underscore the need to enhance measures aimed at personnel hygiene, surface disinfection, and prevention of cross-contamination during processing. The significant presence of *S. aureus* in raw beef and raw chicken samples may point to a need for strengthening hygiene-related controls and improving overall processing conditions.

The high levels of *S. aureus* detected in fish meat and raw milk samples may suggest a possible human-derived contamination during food processing (Table 5).

**Table 5** - *Escherichia coli* and *Staphylococcus aureus* contamination levels (CFU/g or CFU/mL) in raw food samples

| Samples | *Escherichia coli* | | | | | | |
|---|---|---|---|---|---|---|---|
| | $<1.0 \times 10^1$ | $10^1 - <10^2$ | $10^2 - <10^3$ | $10^3 - <10^4$ | $10^4 - <10^5$ | $10^5 - <10^6$ | $10^6 - <10^7$ |
| Raw beef (n = 49) | 22 | 13 | 8 | 3 | 3 | - | - |
| Raw chicken (n = 40) | 22 | 13 | 5 | - | - | - | - |
| Leafy greens (n = 44) | 11 | 12 | 14 | 5 | 2 | - | - |
| Raw milk (n = 45) | 9 | 7 | 8 | 9 | 3 | 6 | 3 |
| Total (n = 178) | 64 | 45 | 35 | 17 | 8 | 6 | 3 |
| **Samples** | ***Staphylococcus aureus*** | | | | | | |
| | $<1.0 \times 10^1$ | $10^1 - <10^2$ | $10^2 - <10^3$ | $10^3 - <10^4$ | $10^4 - <10^5$ | $10^5 - <10^6$ | $10^6 - <10^7$ |
| Raw beef (n = 49) | 41 | 5 | 3 | - | - | - | - |
| Raw chicken (n = 40) | 33 | 4 | 3 | - | - | - | - |
| Fish (n = 42) | 22 | 18 | 2 | - | - | - | - |
| Leafy greens (n = 44) | 30 | 5 | 7 | 2 | - | - | - |
| Raw milk (n = 45) | 24 | – | 4 | 5 | 8 | 2 | 2 |
| Total (n = 220) | 150 | 32 | 19 | 7 | 8 | 2 | 2 |

In raw beef samples, *E. coli* counts ranged from $1 \times 10^1$ to $7.4 \times 10^4$ CFU/g (1 – 4.86 $\log_{10}$ CFU/g), while *S. aureus* counts ranged from $4 \times 10^1$ to $5.6 \times 10^2$ CFU/g (1.60 – 2.74 $\log_{10}$ CFU/g). In raw chicken samples, *E. coli* was detected between $1 \times 10^1$ and $6.4 \times 10^2$ CFU/g (1 – 2.80 $\log_{10}$ CFU/g), and *S. aureus* ranged from $6.1 \times 10^1$ to $8.8 \times 10^2$ CFU/g (1.78 – 2.94 $\log_{10}$ CFU/g). In leafy greens, *E. coli* prevalence was 60% in lettuce, 73.33% in parsley, and 92.85% in spinach, with the highest counts recorded as $2.9 \times 10^4$, $1 \times 10^4$, and $2.8 \times 10^3$ CFU/g, respectively. *S. aureus* was detected in 20% of lettuce, 46.66% of parsley, and 28.57% of spinach samples, with maximum counts of $6.8 \times 10^3$, $3.6 \times 10^2$, and $2.8 \times 10^3$ CFU/g, respectively.

In raw milk samples, *E. coli* counts ranged from $1 \times 10^1$ to $2.4 \times 10^6$ CFU/mL (1 – 6.38 $\log_{10}$ CFU/mL), while *S. aureus* counts varied between $1 \times 10^1$ and $4.4 \times 10^6$ CFU/mL (1 – 6.64 $\log_{10}$ CFU/mL). These findings demonstrate variability in contamination levels of *E. coli* and *S. aureus* across different food groups (Table 5).

In fish meat samples, *E. coli* counts in 17 positive samples ranged from a minimum of 0.74 MPN/g to a maximum of $2.4 \times 10^1$ (2.38 $\log_{10}$) MPN/g. *S. aureus* counts in 20 positive samples ranged from $4 \times 10^1$ (1.60 $\log_{10}$) CFU/g to $3.3 \times 10^2$ (2.51 $\log_{10}$) CFU/g ($p > 0.05$).

According to the Turkish Food Codex Microbiological Criteria Regulation, *Salmonella* spp. and *L. monocytogenes* should not be detected in any product groups. In this context, *Salmonella* spp. was detected in 22 out of 220 samples (10%), and *L. monocytogenes* in two out of 220 samples (0.90%). Among the 22 *Salmonella* positive samples, serotyping identified 20 (90.90%) as *S. enteritidis*, one (4.54%) as *S. typhimurium*, and one (4.54%) as *S. choleraesuis*. Additionally, according to the same regulation, the upper limit for *E. coli* in minced or mechanically separated meat is set at $5 \times 10^2$ CFU/g. In our study, 16.32% (8/49) of raw beef and 5% (2/40) of raw chicken samples exceeded this limit. Furthermore, the regulation sets the upper limit for *E. coli* at $1 \times 10^3$ CFU/g for ready-to-eat chopped fruits and vegetables. Accordingly, 6.66% (1/15) of parsley samples, 20% (3/15) of lettuce and 14.28% (2/14) of spinach samples in our study exceeded this limit. For *S. aureus*, the upper limit is set at $1 \times 10^3$ CFU/g for all ready-to-eat foods, and $1 \times 10^4$ CFU/g for all non-ready-to-eat foods. Accordingly, 6.7% (1/15) of lettuce samples exceeded the limit ($p > 0.05$) (TGK, 2025).

## Discussion

Food safety is a critical element for public health, as illnesses resulting from the consumption of contaminated foods cause significant adverse effects on individuals health and quality of life. Such diseases can affect all segments of society, particularly immunocompromised individuals, the elderly, and chil-

dren, while also imposing substantial economic and logistical burdens on healthcare systems (Wei and Zhao, 2021).

According to WHO, approximately 10% of the global population suffers from foodborne illnesses each year due to the consumption of contaminated food. This situation is especially prevalent in developing countries, where inadequate hygiene practices, unsafe water sources, and deficiencies in food processing contribute to a higher incidence of foodborne diseases, leading to serious public health problems (Cissé, 2019).

In this context, our study examined 220 raw food samples including meat, leafy greens, fish, poultry, and milk for the presence of five major foodborne pathogens: *E. coli*, *Salmonella* spp., *Shigella* spp., *L. monocytogenes*, and *S. aureus*. The results revealed that certain food groups exhibited higher contamination levels, particularly with fecal-origin pathogens. These findings reflect the complex interplay between food type, production practices, and hygiene levels, and highlight the potential for these pathogens to serve as sentinel indicators for guiding future monitoring and intervention strategies.

In this study, the high microbial counts detected in some samples exceeded the limits specified in the Turkish Food Codex and international standards (European Comunity, 2005), indicating potential noncompliance with hygiene regulations. For example, the maximum *E. coli* counts detected in raw milk (6.38 $\log_{10}$ CFU/mL) and raw beef (4.86 $\log_{10}$ CFU/g) are considerably higher than the thresholds generally considered safe for raw food products intended for human consumption. These elevated counts may result from inadequate cleaning of milking equipment, insufficient refrigeration, poor personnel hygiene, or cross-contamination during processing.

The prevalence of *E. coli* in our study (55.10%) is comparable to the findings of Öncül and Yıldırım (2019), who detected *E. coli* in 10 out of 18 raw beef samples in Tokat province. Ncoko et al. (2020) detected *E. coli* in 50% of a total of 150 raw meat samples in South Africa; this value was found to be consistent with the prevalence rates observed in our study. The *E. coli* prevalence rates detected in raw beef samples largely align with findings from studies conducted in different countries. Variations may arise from factors such as sanitation protocols used during animal slaughter, environmental conditions during meat processing, and the effectiveness of the cold chain. Additionally, the risk of intestinal content contaminating the meat during slaughter is directly related to hygiene practices.

In our study, *Salmonella* spp. was detected in 8.16% of raw beef samples. Identification and serotyping of the isolates revealed that all four were *S. Enteritidis*. Similar prevalence rates were reported by Atabey et al. (2021), who detected *Salmonella* spp. in 2.5% out of 120 raw beef samples in Tekirdağ; among these, two isolates were identified as *S. Typhimurium* and one as *S. Bongori*. Gebremedhin et al. (2021) detected *Salmonella* spp. in 20 out of 354 raw beef samples in Ethiopia, with two isolates identified as *S. Typhimurium*. Variations in *Salmonella* spp. prevalence are thought to be influenced by the effectiveness of infection control measures applied in slaughterhouses, the animals' feeding practices, and environmental conditions encountered during transportation. The differing contamination levels observed across regions reflect variability in regional food safety practices.

In our study, *L. monocytogenes* was detected in 2.04% of raw beef samples. Similarly, Pamuk and Siriken (2018) reported a prevalence of 4% *L. monocytogenes* in raw beef samples sold in the Central Aegean Region of Türkiye. Jang et al. (2021) detected *L. monocytogenes* in 0.66% of raw beef samples in their study conducted in South Korea. The presence of *L. monocytogenes* in raw beef is may be influenced by storage and transportation conditions of the meat. Its ability to survive at low temperatures can lead to higher detection rates when cold storage processes are not effectively implemented.

In our study, *S. aureus* was detected in 16.32% of raw beef samples. The S. aureus counts in these eight positive samples ranged from $4 \times 10^1$ (1.60 $\log_{10}$) CFU/g to $5.6 \times 10^2$ (2.74 $\log_{10}$) CFU/g. Öncül and Yıldırım (2019), in a study conducted in Tokat, detected *S. aureus* in 100% of the 18 raw beef samples analyzed, with counts ranging between $2.6 \times 10^3$ and $2.57 \times 10^5$ CFU/g. Similarly, Datta et al. (2012) reported *S. aureus* in 84.21% of raw meat samples in Bangladesh, with an average count of 5.12 $\log_{10}$ CFU/g. *S. aureus* contamination may indicate a possible risk of human-origin transmission, as slaughterhouse personnel can be carriers. Additionally, the level of equipment disinfection during meat processing also influences contamination rates.

In our study, *Shigella* spp. was not detected in any of the raw beef samples analyzed. A similar prevalence rate was reported by Bayram et al. (2011), who found no *Shigella* spp. growth in 40 raw beef samples in Mersin. In contrast, a higher prevalence was observed in a study conducted in Iran, where *Shigella* spp. was detected in five out of 135 raw beef samples; among these, four were identified as *S. sonnei* and one as S. *flexneri* (Pakbin et al., 2021). These differences may be related to factors such as water quality used during slaughter, sanitation of contact surfaces, or potential cross-contamination risks.

In our study, *E. coli* was detected in 45% of raw chicken meat samples. The *E. coli* counts in these 18 positive samples ranged from 1 x $10^1$ (1 $\log_{10}$) to 6.4 x $10^2$ (2.80 $\log_{10}$) CFU/g. A similar prevalence rate was reported by Bonyadian et al. (2011) in Iran, where *E. coli* was detected in 57.27% of raw chicken meat samples. A higher prevalence was observed in a study conducted by Altun and Atasever (2018) in Erzurum, Türkiye, with *E. coli* detected in 76.66% of raw chicken samples; colony counts in positive samples ranged from 1 x $10^1$ to 3.7 x $10^3$ CFU/g, with an average of 2.9 x $10^2$ CFU/g. Variations in *E. coli* detection rates in raw chicken meat across different studies are related to the conditions in which the animals are raised, post-slaughter washing processes, and hygiene standards during transportation. Particularly, live animal transport conditions can significantly influence the spread of this bacterium.

In our study, *Salmonella* spp. was detected in 8.16% of the raw chicken meat samples. As a result of identification and typing, all seven isolates were determined to be *S. Enteritidis*. A similar low prevalence was reported by Yenilmez (2022) in a study conducted in Adana, where *Salmonella* spp. was isolated from 8.33% of raw chicken meat samples. A comparable prevalence rate was observed in a study conducted in Saudi Arabia, where *Salmonella* spp. was detected in 17.57% of 421 chicken meat samples (Saad et al., 2007). The presence of *Salmonella* spp. in raw chicken meat can vary depending on the level of biosecurity in poultry farming environments, the contamination status of feed, and the disinfection procedures applied during slaughtering processes.

In our study, *L. monocytogenes* was detected in 2.5% of the raw chicken meat samples. Among studies reporting higher prevalence rates, Kaya and Atasever (2020) conducted a research in Erzurum and found *Listeria* spp. in 64% of 100 chicken meat samples, with 25% identified as *L. monocytogenes*. In a study carried out in Jordan, *Listeria* spp. was detected in 50.35% of 280 raw poultry products, and 18.21% of these were identified as *L. monocytogenes* (Osaili et al., 2011). The variation in detection rates of *L. monocytogenes* in chicken meat across different studies may be directly related to the adequacy of cold chain practices. Additionally, water sanitation and equipment hygiene in slaughterhouses can significantly influence the spread potential of the bacterium.

In our study, *S. aureus* was detected in 17.5% of the raw chicken meat samples. The *S. aureus* counts in these seven samples ranged from a maximum of 8.8 x $10^2$ CFU/g (2.94 $\log_{10}$) to a minimum of 6.1 x $10^1$ CFU/g (1.78 $\log_{10}$). Among studies reporting similar prevalence rates, Akbar and Anal (2013) found that 18.18% of 209 raw chicken meat samples collected in Thailand were contaminated with *S. aureus*. A higher prevalence was reported by Yıldırım et al. (2015) in a study conducted in Tokat, Türkiye, where a total of 50 raw chicken meat samples, 25 chicken breasts and 25 chicken thighs were analyzed. *S. aureus* was detected in 64% of breast samples and 60% of thigh samples, with average counts of 2.53 x $10^4$ CFU/g and 2.6 x $10^4$ CFU/g, respectively (Yıldırım et al., 2015). The contamination of chicken meat with *S. aureus* can vary depending on the hygiene practices of personnel during processing and the disinfection of contact surfaces. Human-related contamination is one of the most significant factors, especially in meats handled manually.

In our study, *Shigella* spp. was not detected in any of the raw chicken meat samples. Similar prevalence rates were reported by Bayram et al. (2011) in a study conducted in Mersin, where 40 raw chicken meat samples were examined, and none tested positive for *Shigella* spp. Likewise, in a study conducted in Egypt by Ahmed and Shimamoto (2014), *Shigella* spp. was not isolated from any of the 160 raw chicken meat samples analyzed. The absence of *Shigella* spp. may be associated with the effectiveness of water sanitation and disinfection practices applied during poultry processing. In particular, implementing proper hygiene protocols during the evisceration process may help prevent the spread of this bacterium.

In our study, *E. coli* was detected in 40.47% of the fish meat samples. Among these 17 positive samples, the highest count was 2.4 x $10^1$ (2.38 $\log_{10}$) MPN/g, while the lowest was 0.74 MPN/g. A lower prevalence was reported by Pamuk et al. (2019) in a study conducted in Afyonkarahisar, where *E. coli* was detected in 7.31% of 82 fish meat samples, with average levels ranging between 1.30 and 4.47 $\log_{10}ml^{-1}$. In a study conducted in Latvia, *E. coli* was detected in 15% of 20 raw fish meat samples, with bacterial levels ranging from 1.11 to 1.72 $\log_{10}$ CFU/$cm^2$ (Eizenberga et al., 2015). The variability in *E. coli* detection rates in fish meat samples can be attributed to several factors, including the microbiological quality of the water used during fishing and processing, the maintenance of hygienic conditions during transportation, and the storage temperature of the fish.

In our study, *Salmonella* spp. was detected in five of the 42 fish meat samples. Identification and typing of *Salmonella* spp. revealed that three were *S. enteritidis*, one was *S. typhimurium*, and one was *S. choleraesuis*. Among studies with similar prevalence rates, Ikiz et al. (2016) conducted a research in Istanbul and reported *Salmonella* spp. in 12.5% of 400 raw fish meat samples. In a study conducted in Burkina Faso, *Salmonella* spp. was detected in 23.94% of 238 raw fish samples, with *S. bredeney* (8.2%) and *S. colidale* (8.2%) reported as the most common serotypes (Traoré et al., 2015). The presence of *Salmonella* spp. in fish meat is closely related to the contamination level of the water sources where fishing occurs. Additionally, hygienic conditions in processing facilities and the risk of cross-contamination may also contribute to the spread of *Salmonella*.

In our study, *L. monocytogenes* was not detected in any of the fish meat samples. A similar prevalence rate was reported by Telli et al. (2022) in a study conducted in Konya, where none of the 170 raw fish meat samples tested positive for *L. monocytogenes*. A higher prevalence rate than ours was observed in a study conducted by Wieczorek and Osek (2017) in Poland, where *L. monocytogenes* was found in 18.9% of 301 raw fish samples. Due to its ability to survive at low temperatures, *L. monocytogenes* poses a high contamination risk in fish meat. However, the absence of this bacterium in hygienically processed and properly stored fish samples may indicate the implementation of effective food safety measures.

*S. aureus* was detected in 47.61% of the fish meat. Among the positive samples, *S. aureus* counts ranged from a maximum of 3.3 x $10^2$ CFU/g (2.51 $\log_{10}$) to a minimum of 4 x $10^1$ CFU/g (1.60 $\log_{10}$). A lower prevalence rate was reported by Pamuk et al. (2019) in a study conducted in Afyonkarahisar, where a total of 82 raw fish meat samples, 43 gilt-head bream, and 39 sea bass were analyzed, and *S. aureus* was found in 12.19% of them. Detected *S. aureus* levels were reported to range from 1.30 to 6.49 $\log_{10}$ $ml^{-1}$ and from 1.30 to 6.11 $\log_{10}$ $ml^{-1}$, respectively (Pamuk et al., 2019). *S. aureus* contamination may indicate human-related transmission during fish processing. Moreover, the ambient temperature in which the fish are stored and the level of sanitation in processing facilities are also among the key contributing factors.

In our study, *Shigella* spp. was not detected in any of the fish meat samples. Tosun et al. (2016) conducted a research in Istanbul and found *S. dysenteriae* in 3.05% and *S. sonnei* in 3.66% of 124 fish meat samples. In a study by Obaidat et al. (2017), *Shigella* spp. was detected in 19% of 330 raw fish meat samples. The absence of *Shigella* spp. in our study may be associated with the microbiological quality of the waters where the fish were caught and the effectiveness of sanitation procedures implemented in the processing facilities.

In our study, *E. coli* was detected in 75% of the leafy green samples. Among studies reporting lower prevalence rates, Alçay (2021) conducted a research in Istanbul and found *E. coli* in only 10% of 30 raw vegetable samples (tomato, carrot, lettuce, onion, and parsley), with detected levels ranging from <10 to 2 x $10^1$ CFU/g (<1 – 1.30 $\log_{10}$). Among studies reporting prevalence rates similar to ours, a study conducted in Brazil examined 162 semi-processed leafy greens (kale, lettuce, parsley, spinach) and detected E. *coli* in 53.08% of the samples (Oliveira et al., 2011). The high detection rate of *E. coli* in leafy green samples may be associated with the quality of irrigation water used in agricultural production, soil characteristics, and post-harvest processing practices.

In our study, *Salmonella* spp. was detected in 6.81% of the leafy green samples. Identification and typing of *Salmonella* spp. revealed that all three isolates – one from parsley and two from spinach – were *S. Enteritidis*. Among studies with similar findings, a study conducted in Istanbul examined 30 raw vegetable samples and detected *Salmonella* spp. in only 3.33% of them (Alçay, 2021).

In a study carried out by Badosa et al. (2008) in Spain, *Salmonella* spp. was found in 0.67% of the 445 raw and ready-to-eat vegetable samples analyzed. The occurrence of *Salmonella* spp. in leafy green samples may be attributed to improper treatment of organic fertilizers used in the field or the use of contaminated water in agricultural irrigation.

In our study, *L. monocytogenes* was not detected in any of the leafy green samples. A similar prevalence rate was reported by Kara et al. (2019) in a study conducted in Afyonkarahisar, where 70 fresh lettuce samples were analyzed and *L. monocytogenes* was found in only 1.42% of them. In contrast, a study conducted by Jamali et al. (2013) in Malaysia examined 145 raw vegetable samples (carrot, cabbage, parsley, and cucumber) and reported that *L. monocytogenes* was isolated from 35.17% of the samples. The varying detection rates of *L. monocytogenes* across different studies may be explained by differences in climate conditions, agricultural production techniques, and storage processes.

In our study, *S. aureus* was detected in 31.81% of leafy green samples. Studies with similar prevalence rates include one conducted in Istanbul (Alçay, 2021), where 30 raw vegetable samples were analyzed, and *S. aureus* levels were reported as <10 – 3.4 x $10^2$ CFU/g (<1 – 2.53 $\log_{10}$). Similarly, Jia et al. (2024) examined 77 raw vegetable samples and detected *S. aureus* in 31.16% of them. The presence of *S. aureus* in leafy greens highlights the risk of human-associated contamination during post-harvest handling. In particular, inadequate surface hygiene during packaging and transportation may contribute to the spread of this bacterium.

In our study, *Shigella* spp. was not detected in any of the leafy green samples. Similar prevalence rates were reported by Çetinkaya et al. (2008), who analyzed 78 raw vegetable and 100 salad samples in Bursa, Türkiye, and found no presence of *Shigella* spp. In contrast, a higher prevalence was reported by Shahin et al. (2019) in Iran, where 650 raw vegetable samples were examined and *S. sonnei* was identified in 1.23% and *S. flexneri* in 0.92% of the samples. The absence of *Shigella* spp. in our study may be associated with the cleanliness of irrigation water and the adequacy of hygienic practices during production. However, the low prevalence reported in some studies indicates that the risk of human-associated contamination cannot be entirely ruled out.

In our study, *E. coli* was detected in 80% of raw milk samples. The E. coli counts in these samples ranged from 1 x $10^1$ (1 $\log_{10}$ CFU/mL) to 2.4 x $10^6$ (6.38 $\log_{10}$ CFU/mL). This prevalence is considerably higher compared to previous studies. For example, in a study conducted by Tuncay et al. (2022) in Van, Türkiye, *E. coli* was found in 35% of raw milk samples, with bacterial loads ranging from <1 to 3.52 $\log_{10}$ CFU/mL. Similarly, in a study conducted in Tunisia, *E. coli* was identified in 32.5% of the raw milk samples, with counts ranging from 2.08 ± 0.11 to 2.69 ± 0.12 $\log_{10}$ CFU/mL (Bali et al., 2013). The high prevalence and contamination levels observed in our study may indicate possible inadequacies in milking hygiene. This situation may be influenced by factors such as possible inadequacies in sanitation of milking equipment, poor personal hygiene of farm workers, suboptimal housing conditions of animals, and inadequate cleaning of milk collection containers. Furthermore, since *E. coli* is a fecal indicator microorganism, its presence in raw milk reflects fecal contamination of animal origin and poses a serious risk to public health.

In our study, *Salmonella* spp. was detected in 6.81% of raw milk samples. Serotyping and identification revealed that all three isolates belonged to *S. Enteritidis*. The low prevalence observed in our study aligns with the findings of Tuncay et al. (2022), who reported no detection of *Salmonella* spp. in 60 raw milk samples collected in Van, Türkiye. A comparable prevalence was reported by Kaushik et al. (2014) in a study conducted in India, where 142 raw milk samples were analyzed, and *Salmonella* spp. was detected in 3.52% of the samples. Among the five isolates identified, three were classified as *S. typhimurium*, and two as *S. newport*. The presence of *Salmonella* spp. in raw milk may be associated with several factors, including the animals' dietary habits, barn hygiene, and the effectiveness of control measures implemented during post-milking processing stages.

In our study, *L. monocytogenes* was not detected in any of the raw milk samples. Among studies reporting higher prevalence rates, Aksoy et al. (2018) conducted a research in Kars, where 100 raw milk samples were examined. *Listeria* spp. was detected in 10% of the samples, and a total of 26 isolates were obtained from these 10 samples. Of the isolates, 16 were identified as *L. monocytogenes*.

In another study conducted in Egypt, 103 raw milk samples were analyzed, and *L. monocytogenes* was detected in 1.94% of the samples (Osman et al., 2016). The absence of *L. monocytogenes* in some studies may be associated with the rapid processing and storage of raw milk under cold chain conditions. In our study, *S. aureus* was detected in 53.33% of the raw milk samples. Among these 21 positive samples, the *S. aureus* count ranged from a maximum of $4.4 \times 10^6$ CFU/mL (6.64 $\log_{10}$) to a minimum of $1 \times 10^1$ CFU/mL (1 $\log_{10}$). A lower prevalence rate than in our study was reported by Şimşek (2021), who examined 10 raw milk samples and did not detect *S. aureus* in any of them. In contrast, a higher prevalence was observed in a study conducted by Tankoano et al. (2016) in Burkina Faso, where *S. aureus* was detected in all 45 raw milk samples, with an average *S. aureus* count of 4.78 $\log_{10}$ CFU/mL. The presence of *S. aureus* in raw milk samples may result from direct contact during milking with contaminated hands, equipment, or the animal's skin.

In our study, *Shigella* spp. was not detected in any of the raw milk samples. Similarly, in a study conducted by Pakbin et al. (2021), *Shigella* spp. was not detected in any of the 135 raw milk samples analyzed. In the study conducted by Shahin et al. (2019), *Shigella* spp. was not detected in any of the 100 raw milk samples analyzed. The absence of *Shigella* spp. may be attributed to the low risk of cross-contamination during milking and the fact that this bacterium is not typically present in the intestinal flora of dairy animals.

The presence of bacterial pathogens in raw food samples has been discussed in detail, highlighting how it may vary across different geographical regions and under varying hygienic conditions. In our study, *E. coli* contamination was found to be statistically significant, particularly in fish meat, leafy greens, and raw milk samples. Similarly, the presence of *S. aureus* was determined to be significant in raw beef, raw chicken, fish meat, and raw milk samples. These results are consistent with data reported in previous studies and underscore the public health risks associated with such food products. The findings suggest that microbial contamination in food products may show regional variations, potentially influenced by multiple factors such as milking equipment cleanliness, personnel practices, processing conditions, environmental contamination, and local production systems.

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These findings should also be interpreted in the context of relevant food safety legislation. The high microbial loads observed in several sample types suggest that hygiene control measures along the production-to-consumption chain may be insufficient, necessitating stricter adherence to national and international standards. Comparisons with regulatory limits underline the need for immediate corrective actions to ensure compliance and reduce public health risks.

This study was conducted with raw food samples collected within a specific time frame (May–October 2024) and limited to the Kocaeli province, which may restrict the ability of the data to fully capture seasonal and regional variations. Nevertheless, the sample size was determined using epidemiological estimation methods (expected prevalence, 95% confidence level, and 5% margin of error), ensuring statistically reliable prevalence data for the studied population. All microbiological analyses were performed according to internationally validated ISO protocols harmonized with Codex Alimentarius and the Turkish Food Codex, providing methodological rigor and regulatory relevance. While these approaches offer robust baseline data, larger multi-provincial and longitudinal studies are needed to strengthen the generalizability of the findings. Furthermore, incorporating complementary molecular techniques in future research could enhance the confirmation, subtyping, and epidemiological tracking of foodborne pathogens.

## Conclusion

This study revealed the presence of bacterial pathogens in raw food products, assessing the associated microbiological risks and their implications for public health. The levels of contamination detected in different food groups highlight potential gaps in hygiene-related practices and other possible

contributing factors along the production-to-consumption chain. The high microbial counts observed in several food types exceed regulatory thresholds, emphasizing the need for improved hygiene, proper storage, and adherence to legal standards.

In raw meat products, potential contamination may be related to factors such as sanitation conditions during slaughtering and processing, environmental contamination, or handling practices; in leafy greens, contamination risks may be associated with irrigation water quality, production conditions, or post-harvest handling practices; and in raw milk samples, potential influencing factors may include milking equipment cleanliness, animal health status, environmental conditions, and handling practices.

These findings underscore the need for stricter implementation of national and international hygiene standards and the strengthening of effective control mechanisms at every stage of the food supply chain. To reduce the risks associated with the consumption of raw food, it is essential to enhance control measures from production to consumption and to expand awareness efforts targeting both producers and consumers. Future research could contribute to a better understanding of contamination sources by examining the genetic characteristics of pathogens detected in raw foods.

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## Authors´contributions

AS was responsible for visualization and writing of the original draft. DD, for the project administration. DKE and DD, for supervision, writing review and editing. All authors contributed to the data curation, investigation, methodology, and approval of the final version.

## Data availability statement

The research data are not publicly available.

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