

## Microbiological hazards and compliance of ready-to-eat foods at restaurants..

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### ABSTRACT

This study aimed to evaluate the microbiological hazard of 210 ready-to-eat (RTE) food samples collected from local markets in Jordan, including salads, falafel sandwiches, shawerma sandwiches, and garlic sauces. Microbial analysis including *Escherichia* (E) coli, *Bacillus* (B) cereus, *Staphylococcus* (S) aureus, *Listeria* (L) monocytogenes, and *Salmonella* spp., was performed using ISO methods. While *Salmonella* spp. and *L. monocytogenes* were absent in all samples, *E. coli*, *B. cereus*, and *S. aureus* were detected in 16.2%, 15.2%, and 5.7% of samples, respectively. The highest microbial load was in falafel sandwiches and salads, with some samples exceeding Jordanian microbiological safety limits. The presence of *E. coli* indicates possible fecal contamination, whereas *B. cereus* and *S. aureus* suggest cross-contamination and poor hygiene during preparation and handling of food samples. These findings highlight the potential public health risks associated with RTE foods in Jordan, particularly from informal and small-scale restaurants and canteens.

**Keywords:** Food safety, Microbiological hazards, Ready-to-eat food, Restaurants, pathogenic bacteria

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### 1. INTRODUCTION

Food safety is a critical pillar of public health, particularly in developing and middle-income countries where foodborne illnesses remain widespread and under-regulated (Grace, 2015, Grace, 2023). The World Health Organization estimates that approximately 600 million people fall ill annually due to the consumption of contaminated food, resulting in 420,000 deaths, including 125,000 children under the age of five ((WHO, 2024)). Among the most significant contributors to this burden are microbiological hazards, primarily caused by pathogenic microorganisms such as *Salmonella* spp., *Escherichia* (E) coli, *Listeria* (L) monocytogenes, and *Staphylococcus* (S) aureus (Newell et al., 2010, Janecko et al., 2023). Foodborne pathogens have always been of public health concern and represent safety issues for food consumers and official authorities. These pathogens can lead to a wide spectrum of illnesses, ranging from self-limiting gastroenteritis to life-threatening systemic infections, particularly in immunocompromised individuals and vulnerable populations (Mantovam et al., 2025)

In many low- and middle-income countries, including Jordan, local restaurants, and informal street food vendors serve as a primary source of affordable, ready-to-eat meals. These food establishments, often operating with minimal oversight, are embedded within communities due to their accessibility, cultural relevance, and economic feasibility. Common food items, such as meat-based dishes, rice, legumes, pasta, dairy products, salads, sandwiches, juices, and baked goods, are frequently prepared under substandard sanitary conditions. Those local restaurants often work in settings that lack access to basic infrastructure, including clean running water, refrigeration, proper waste disposal, and handwashing facilities. As reported in studies from similar settings (Barro et al., 2006, Muyanjanja et al., 2011), these operational deficiencies contribute to the high risk of microbial contamination.

Contributing factors include inadequate food safety knowledge, poor personal hygiene, the reuse of contaminated utensils, lack of access to resources, and the use of untreated water during food preparation and cleaning (Mosimann et al., 2023, Alrabadi et al., 2013). Furthermore, food is often exposed to ambient temperatures for extended periods and may be subject to cross-contamination between raw and cooked ingredients—both of which facilitate microbial growth and increase the risk of foodborne disease outbreaks (Ehiri et al., 2001).

Assessing the microbiological quality of food sold in such environments is essential to understanding the extent of

contamination and informing control strategies. Methods such as total viable count (TVC), coliform enumeration, and culture-based or molecular detection of specific pathogens provide valuable insights into food hygiene and safety. These evaluations are not only critical for consumer health but also serve as the foundation for evidence-based interventions, including hygiene training, policy enforcement, and the application of Hazard Analysis and Critical Control Points (HACCP) principles (WHO, 2022, CAC, 2003).

Various studies have been conducted on the contamination with bacteria in Ready-To-Eat (RTE) foods in different countries and different results have been reported.

In Korea, a total of 634 samples were collected from the local market. The aerobic plate count levels of was 1.0–7.9 log CFU/g. *E. coli* and *Listeria monocytogenes* were detected in only 2 samples in the qualitative test. *S. aureus* and *B. cereus* were detected in 12.3 and 12.6% of the samples at levels up to 1 log CFU/g.

A study was conducted to detect *Salmonella* and *Escherichia coli* contamination and total microbial count in ready-to-eat salad samples containing meat products in Tehran in 2018. The microbial analysis of 136 samples, including salads, was done according to the ISO international standards. *Salmonella* was not detected in any of the samples, and only 0.7% of the samples were contaminated with *E. coli* (Koushki et al., 2021).

In another study, samples of four RTE salads, tabbouleh, hummus, Greek salad, and coleslaw were contaminated with *E. coli* but not with *S. aureus*, *Salmonella*, or *L. monocytogenes* (Almualla et al., 2010).

In Jordan, a study examined the microbial quality of tabbouleh, a popular Middle Eastern salad. The results showed that, the local salads have higher counts of bacteria indicating improper hygiene procedures during salads preparation (Kidar and Yamani, 2004).

The Jordan Food and Drug Administration classified the food based on food quality and safety to four categories: highly safe, accepted, not accepted, and critical (Table 1). The classification depends on the type and number of specific microbes (JFDA, 2011).

**Table (1) food quality classification based on Jordanian standard.**

Parameter	Highly safe	accepted	Not accepted	critical
<i>E. coli</i>	<10	10-100	>100	have pathogenic <i>E. coli</i>
<i>Salmonella spp</i>	Absent			Present
<i>Bacillus (B) cereus</i>	100	100-1000	1000-10000	>10000
<i>L. monocytogenes</i>	<10		10-100	>100
<i>S. aureus</i>	100	100-1000	1000-10000	>10000

Even though there are some researchers who have been studied the microbial quality of RTE, the real situation in Jordan is not controlled, and more studies are needed to understand the current situation and how to assess the risk behind that. Therefore, this study aims to investigate the microbial safety of selected popular food items sold by local eateries and street vendors across various Jordanian localities. By identifying contamination levels and potential sources, the study seeks to support public health strategies aimed at enhancing food safety, reducing foodborne illness, and informing national food policy.

## 2. MATERIALS AND METHODS:

### 2.1 Samples Collection

Overall, 204 ready-to-eat salads were collected from the local restaurants in Jordan. They included 52 salad samples, 52 falafel and humus sandwiches, 50 shawerma sandwiches, and 50 garlic sauce samples. Then they were transported to the laboratory in an icebox and refrigerated until the microbial tests were conducted. All salads were packed in sterile containers and ranged from 200 to 500 grams.

### 2.2 Microbial Analysis

Five microbial tests, including *E. coli*, *B. cereus*, *S. aureus*, and *L. monocytogenes* enumeration, and *Salmonella spp* detection, were conducted according to the methods recommended by the ISO International Standard.

### 2.2.1 *E. coli* enumeration

Twenty-five grams of each sample were added to 225 mL of buffered peptone water. After that, one mL from the initial suspension and from further dilution was added to each of two sterile Petri dishes. Pouring 15 ml of tryptone-bile-glucuronic medium into each Petri dish previously cooled to 44-47 in a water bath, followed by incubation of plates in an inverted position at  $44 \pm 1^\circ\text{C} \pm 1^\circ\text{C}$  for 18 hrs to 24 hrs. After 24 hrs the blue colonies counted as the *E. coli* confirmed colonies (ISO, 2001)

### 2.2.2 *Salmonella* spp. detection

Twenty-five grams of each sample was added to 225 mL of buffered peptone water. After homogenization, the samples were incubated at  $37^\circ\text{C}$  for 18-24 hrs, followed by selective enrichment in Rappaport- Vassiliadis medium with soya (RVS) broth at  $41.5^\circ\text{C}$  for 24 hrs and Muller-Kauffmann tetrathionate-novobiocin (MKTn) broth at  $37^\circ\text{C}$  for 24h. Then the Xylose Lysine Deoxycholate (XLD) agar and brilliant green agar plates were inoculated with the enriched cultures obtained from the RVS and MKTn broths and incubated at  $37^\circ\text{C}$  for 24 hrs. Typical isolated colonies on the XLD and Hekton enteric agar plates were further confirmed using biochemical tests by inoculating in Triple Sugar Iron (TSI) agar slope, Urea Agar, L-Lysine Decarboxylation (LDC) medium, and tryptone water for the indole test (ISO., 2017)

### 2.2.3 *B. cereus* enumeration

Twenty-five grams of each sample was added to 225 mL of buffered peptone water. After that, 1 ml from the initial suspension and from further dilutions was distributed to three sterile Petri dishes having mannitol egg yolk polymyxin agar. The plates were incubated at  $30 \pm 1^\circ\text{C}$ . for 8 to 24 hrs. the suspected colonies (pink colonies) were counted and confirmed by showing hemolysis on sheep blood agar (ISO, 2004).

### 2.2.4 *L. monocytogenes* enumeration

Twenty-five grams of each sample were added to 225 mL of half Fraser broth, and was incubated at  $30^\circ\text{C}$  for 24 hrs. After that, 0.1 from the incubated broth was transferred to Fraser broth and was incubated at  $37^\circ\text{C}$  for 24 hrs. From the Fraser broth, *Listeria* Chromogenic agar and oxford agar were inoculated and incubated at  $37^\circ\text{C}$  for 24 hrs. The suspected colonies were confirmed with motility tests, carbohydrate utilization (rhamnose and xylose), and the CAMP test (ISO, 2017).

### 2.2.5 *S. aureus* enumeration

Three Baird Parker agar were inoculated with 1 ml of the initial dilution and further dilution. The plates were incubated at  $37^\circ\text{C}$  for 48 hrs. The suspected colonies were confirmed using the coagulase agglutination test (ISO, 1999).

## 3. RESULTS

A total of 210 ready-to-eat samples including 52 salad samples, 52 falafel and humus sandwiches, 50 shawerma sandwiches, and 50 garlic sauces samples were tested for *E. coli*, *B. cereus*, *S. aureus*, and *L. monocytogenes* enumeration, and *Salmonella* spp. detection. The results showed that few samples have *E. coli*, *B. cereus*, and *S. aureus*, but none of the samples contaminated with salmonella or *L. monocytogenes*.

Table 2 presents the results of *E. coli* enumeration. *E. coli* was detected in 12 salad samples, with 8 meeting Jordanian standards and 4 considered not acceptable. In falafel sandwich samples, *E. coli* was found in 17 samples, of which 9 were acceptable and 8 were not. Within shawerma sandwich samples, 5 contained *E. coli*; 4 were considered acceptable, and 1 was not. *E. coli* was not detected in any of the garlic sauce samples.

**Table 2: The results of *E. coli* enumeration.**

	<i>E. coli</i>		
samples	<10	10- 100	>100
Salad	40	8	4
falafel sandwich	35	9	8
shawerma sandwich	45	4	1
garlic sauce	50	0	0

*B. cereus* was detected in 32 food samples, including 6 salad samples, of which 5 were acceptable and 1 was not accepted. In 15 falafel sandwich samples, 5 were acceptable, 2 were not accepted, and 5 were classified as critical. Among 7 shawarma sandwich samples, 5 were within acceptable limits and 2 were not accepted. Moreover, 4 samples of garlic sauce

showed the presence of *B. cereus*, with 3 being acceptable and 1 not accepted (Table 3).

**Table 3: The results of *B. cereus* enumeration**

	<i>B. cereus</i>			
samples	100	100- 1000	1000-10000	>10000
Salad	45	5	1	0
falafel sandwich	5	2	5	3
shawerma sandwich	43	5	2	0
garlic sauce	46	3	1	0

*S. aureus* was detected only in salad and falafel sandwich samples. In salads, 5 samples were contaminated, with 4 samples being acceptable and one not being acceptable. Among falafel sandwich samples, 7 samples were found contaminated, 5 were acceptable, and 2 samples were not acceptable (Table 4).

**Table 4: The results of *S. aureus* and *L. monocytogenes* enumeration, and *salmonella spp.* detection.**

	<i>S. aureus</i>				<i>Salmonella spp</i>		<i>L. monocytogenes</i>		
samples	<100	100-1000	1000-10000	>10000	Absent	Present	<10	10-100	>100
Salad	47	4	1	0	51	0	51	0	0
falafel sandwich	45	5	2	0	52	0	52	0	0
shawerma sandwich	50	0	0	0	50	0	50	0	0
garlic sauce	50	0	0	0	50	0	50	0	0

#### 4. DISCUSSION

The findings of this study highlight significant concerns about the microbiological safety of some RTE foods commonly sold by local restaurants in Jordan. While *Salmonella spp.* and *L. monocytogenes* were not detected in any of the 210 tested samples, the contamination with *E. coli*, *B. cereus*, and *S. aureus* was evident across multiple food categories, underscoring gaps in hygiene and food handling practices.

Table 2 presents the results of *E. coli* enumeration. The detection of *E. coli* in 34 samples (16.2%), with 13 exceeding the acceptable threshold of 100 CFU/g based on Jordanian standards (JFDA, 2011) is considered a strong indicator of fecal contamination or poor sanitary conditions during food preparation. Remarkably, falafel sandwiches exhibited the highest *E. coli* counts, followed by salads and shawerma sandwiches. This aligns with prior studies that indicate RTE vegetable-based and mixed-ingredient foods are especially susceptible to contamination due to frequent handling (Kidar and Yamani, 2004; Barro et al., 2006; Muyanja et al., 2011).

This findings are in line with results from Tehran, where *E. coli* was detected in 0.7% of salad samples (Koushki et al., 2021), and from the UAE, where *E. coli* was found in various RTE foods but not *S. aureus*, *Salmonella*, or *L. monocytogenes* (Almualla et al., 2010). Although, the prevalence in Jordan appears higher, the absence of *Salmonella* and *L. monocytogenes* in the current study is similar to findings from Korea and other international studies, where these pathogens were rarely or not detected (Cho et al., 2011).

*B. cereus* was found in 32 samples (15.2%), including critical levels (>10,000 CFU/g) in some falafel samples (Table 3). As a spore-forming bacterium, *B. cereus* can survive inadequate cooking and proliferate when foods are held at unsafe temperatures (FSA, 2019; FAO and WHO, 2009). The high prevalence in falafel and shawerma sandwiches may reflect temperature abuse or improper storage, conditions that were widely claimed in previous studies (Barro et al., 2006,

Mosimann et al., 2023).

As shown in table 4, the detection of *S. aureus* in 12 samples, particularly in salads and falafel sandwiches, suggests post-processing contamination, likely through direct contact with food handlers. The low-to-moderate levels observed (mostly <1,000 CFU/g) suggest poor personal hygiene, a factor repeatedly identified as a contributor to foodborne illness risk in low-resource settings (Mosimann et al., 2023, Muyanja et al., 2011, Ehiri et al., 2001). While levels did not exceed the critical threshold (>10,000 CFU/g), the presence of *S. aureus* poses concern and may lead to toxin production, especially under conditions that support.

Although the absence of *Salmonella* and *L. monocytogenes* is encouraging, their potential occurrence in other untested seasons or locations cannot be ruled out. Variations in food handling, water quality, and ingredient sourcing across time and regions may contribute to intermittent contamination (Mantovam et al., 2025, Newell et al., 2010).

Based on the Jordanian food safety classifications (JFDA, 2011), a significant proportion of tested samples fell outside the "highly safe" and "accepted" categories. This finding suggests regulatory enforcement is either lacking or inconsistently applied, particularly in informal food sectors where control on food production is minimal. Given that these establishments often serve economically vulnerable populations, the associated public health risks may be amplified.

Finally, the results of this study confirm the critical importance of implementing and enforcing food safety interventions. These may include structured hygiene training for food handlers, public awareness campaigns, HACCP-based food control systems, and improved sanitation infrastructure, based on Codex Alimentarius and WHO guidelines (CAC, 2003, WHO, 2022).

## 5. CONCLUSION

The microbiological assessment of RTE foods in Jordan revealed that considerable samples are contaminated with *E. coli* and *B. cereus*, representing the most frequent hazards. The absence of *Salmonella* spp. and *L. monocytogenes* is encouraging; however, the detection of *S. aureus* and high levels of *E. coli* in some samples indicate poor hygiene practices during preparation and handling. Falafel sandwiches and salads were the most contaminated categories, suggesting frequent manual handling may increase the risk. These results emphasize the necessity for enforcing food safety regulations, implementing HACCP-based systems, and providing continuous hygiene training for food handlers. Future studies should include broader seasonal and geographical sampling to better assess contamination trends and guide national food safety policies.

## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript..

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