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Surveillance of Foodborne Pathogens in Non-Heated Foods in Seoul, 2021–2024

Young Eun Kim [1,*], Young-Hee Choi [2], Jaekyoo Lee [1], Sohyeon Park [1], Boram Kwak [1], Jiyoon Yeon [1], Hyunjeong Kim [1], Ouk-Hee Kim [2] and Sujin Jeon [1]

[1] Food and Drug Department, Seoul Metropolitan Government Research Institute of Public Health and Environment, Gwacheon 13818, Republic of Korea
[2] Department of Infectious Disease, Seoul Metropolitan Government Research Institute of Public Health and Environment, Gwacheon 13818, Republic of Korea
* Correspondence: imbelo7@seoul.go.kr

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Abstract: Non-heated foods pose an increased risk of foodborne illness because they bypass a final lethal heat treatment. This surveillance study summarizes routine monitoring data on foodborne pathogens in food-service establishments in Seoul, Republic of Korea, from 2021 to 2024. A total of 7683 food samples were collected by 25 public health centers, including 1570 non-heated foods categorized as animal-, fishery-, or agricultural-derived. Samples were tested for bacterial and viral foodborne pathogens in accordance with national guidelines issued by the Ministry of Food and Drug Safety (MFDS). Seven bacterial pathogens were detected among the targeted organisms in non-heated foods, with an overall detection rate of 3.06% (48/1570; 95% CI: 2.31–4.03%), exceeding that observed in heat-treated foods (1.23%; 95% CI: 0.99–1.54%). *Bacillus cereus* and *Vibrio parahaemolyticus* were the most frequently identified pathogens. Eight non-heated food samples were classified as non-compliant according to MFDS microbiological criteria, including zero-tolerance detections (enterohemorrhagic *Escherichia coli*, *Yersinia enterocolitica*, and *Vibrio vulnificus*) and exceedances of quantitative limits (*Staphylococcus aureus* and generic *Escherichia coli*). Detection rates were highest in fishery-derived foods (18.18%; 95% CI: 11.15–28.24%) and animal-derived foods (11.63%; 95% CI: 5.07–24.48%), compared with agricultural-derived foods (2.00%; 95% CI: 1.39–2.86%). No *Salmonella spp.* or enteric viruses were detected in non-heated foods during the study period. These findings support prioritization of non-heated animal- and fishery-derived foods within risk-based surveillance frameworks and reinforce the importance of cold-chain management and cross-contamination control in urban food-service environments.

Keywords: foodborne pathogens; non-heated foods; ready-to-eat foods; microbial contamination; food safety surveillance

1. Introduction

Foodborne diseases remain a significant global public health issue, with ready-to-eat foods posing particular concern due to the absence of a final heat treatment prior to consumption [1]. Non-heated foods, which are served ready for consumption without cooking at the point of service, may facilitate the survival and transmission of foodborne pathogens introduced during raw material handling, preparation, or distribution [2].

Global burden estimates indicate that hundreds of millions of foodborne illness cases occur annually, with bacterial pathogens such as *Salmonella spp*., *Bacillus cereus*, *Staphylococcus aureus*, and pathogenic *Escherichia coli* contributing substantially to morbidity [1,3]. Surveillance studies have consistently reported higher

contamination risks in animal- and fishery-derived ready-to-eat foods compared with plant-based products, reflecting differences in intrinsic microbial ecology and processing environments [4,5].

In recent years, urban food-service settings have received increasing attention as critical control points for food safety management. Studies from East Asia have demonstrated that food handling practices, cold-chain control, and cross-contamination during preparation are key determinants of microbial contamination in non-heated foods served in restaurants and institutional catering facilities [6,7]. Furthermore, foodborne viruses, although often under-detected in routine monitoring, remain epidemiologically important due to their low infectious dose and environmental persistence [8].

In the Republic of Korea, municipal-level surveillance programs conducted by public health authorities play a central role in monitoring microbiological safety in food-service establishments. However, comprehensive multi-year analyses focusing specifically on non-heated foods served in urban environments remain limited. Therefore, this study aimed to investigate the prevalence and regulatory non-compliance of foodborne pathogens in non-heated foods collected from food-service establishments in Seoul from 2021 to 2024, providing evidence to support risk-based surveillance and targeted hygiene interventions.

2. Materials and Methods

2.1. Study Design and Food Sample Collection

This study was conducted within a legally mandated food safety surveillance program operated by the Seoul Metropolitan Government Research Institute of Public Health and Environment (SIHE), Republic of Korea. Between January 2021 and December 2024, a total of 7683 food samples were collected from food-service establishments through 25 local public health centers in Seoul as part of routine microbiological monitoring.

Food-service establishments included restaurants, institutional food services (e.g., school cafeterias), and catering facilities. Establishments were inspected through routine rotational surveillance and risk-based targeting according to municipal inspection plans. Samples were classified as non-heated foods (prepared and served without thermal processing at the point of service) or heat-treated foods. Of the total samples, 1570 non-heated food samples were included in the present analysis. Non-heated foods were further categorized into animal-derived foods (n = 43), fishery-derived foods (n = 77), and agricultural-derived foods (n = 1450), including salads, fresh-cut produce, and pickled vegetables such as kimchi. Sampling was conducted throughout the year without intentional restriction by season.

Samples were aseptically collected, transported under refrigerated conditions (≤4 °C), and analyzed within 24 h of collection. Each food sample was treated as an independent analytical unit in accordance with standard surveillance practice.

As this investigation was conducted as part of routine public health surveillance, it was exempt from institutional review board approval under national regulations.

2.2. Target Foodborne Pathogens and Analytical Methods

Microbiological analyses targeted bacterial and viral foodborne pathogens selected according to the Guideline for Standardization of Food Poisoning Investigation [9] and the MFDS Food Code: Microbiological Test Methods (2024 edition) [10]. Target bacterial pathogens included *Listeria monocytogenes*, *Bacillus cereus*, pathogenic *Escherichia coli* (EAEC, EIEC, EPEC, EHEC, ETEC), *Salmonella spp., Shigella spp., Yersinia enterocolitica, Staphylococcus aureus, Vibrio spp., Campylobacter jejuni*/*coli,* and *Clostridium perfringens*. Generic *Escherichia coli* was treated as a hygiene indicator organism rather than a foodborne pathogen. Viral pathogens included norovirus GI and GII, adenovirus, astrovirus, sapovirus, and rotavirus.

All listed pathogens represent organisms routinely monitored under the national surveillance framework; only pathogens detected during the study period are reported in the Results section.

Sample preparation, culture-based detection, and molecular screening were performed in accordance with the MFDS Food Code: Microbiological Test Methods and the Guideline for Standardization of Food Poisoning Investigation. For bacterial pathogens, 25 g (or mL) of each sample was subjected to organism-specific enrichment and selective culture, followed by biochemical confirmation and automated identification according to MFDS protocols. Molecular screening using multiplex real-time PCR assays was applied where appropriate to support rapid detection. Detailed analytical procedures, including enrichment conditions, selective media, incubation parameters, and confirmation workflows, are summarized in Supplementary Table S2. Corresponding MFDS microbiological criteria, measurement basis (qualitative vs. quantitative), and definitions of non-compliance are presented in Supplementary Table S3 to ensure transparency and regulatory consistency.

For viral pathogens, sample pretreatment and concentration procedures were adapted according to food matrices (e.g., fresh vegetables, pickled foods, and meat products) following MFDS Food Code protocols for foodborne virus detection. Viruses were concentrated using polyethylene glycol (PEG 8000) precipitation combined with differential centrifugation. Viral RNA was extracted using a validated commercial extraction kit (QIAamp Viral RNA Mini Kit, Qiagen, Hilden, Germany), with extraction controls included in each batch. Detection of norovirus GI/GII, adenovirus, astrovirus, rotavirus, and sapovirus was performed using multiplex real-time RT-PCR assays (Kogene Biotech, Seoul, Korea) in accordance with manufacturers' instructions and MFDS guidelines. Samples were considered positive when amplification curves met the cycle threshold (Ct) criteria specified in the national protocol, and positive results were confirmed by conventional RT-PCR and sequencing. Positive, negative, and extraction controls were included in each analytical run to ensure assay validity.

Supplementary Table S2 summarizes the analytical procedures applied to all bacterial pathogens included in the surveillance framework during 2021–2024. Viral pathogens are not included in Supplementary Table S2 because no viral detections occurred during the study period and regulatory non-compliance was not applicable.

Supplementary Table S3 presents the MFDS microbiological criteria only for organisms that were classified as non-compliant in this study (enterohemorrhagic *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio vulnificus*, *Staphylococcus aureus*, and generic *Escherichia coli*), including their measurement basis and regulatory thresholds.

2.3. Serotyping and Data Interpretation

Serotyping of *Salmonella, Escherichia coli, Vibrio*, and *Shigella* isolates was performed using commercial antisera according to manufacturers' instructions. Microbiological results were interpreted according to MFDS regulatory criteria (Supplementary Table S3). For pathogens subject to zero-tolerance standards, non-compliance was defined as detection in any test portion (presence in 25 g). For organisms with quantitative limits, non-compliance was defined as measured values exceeding the category-specific threshold (CFU/g). Regulatory criteria for organisms not detected or not exceeding thresholds are established in the MFDS Food Code but are not reproduced in Supplementary Table S3 to avoid redundancy.

2.4. Statistical Analysis

Each food sample was treated as an independent analytical unit. Because sampling was conducted within a routine municipal surveillance framework, repeat sampling from the same establishment may have occurred; therefore, clustering at the establishment level was not modeled. The analyses are descriptive in nature. Detection rates were calculated as proportions with 95% confidence intervals (CIs) estimated using the Wilson score method. Differences in detection rates between non-heated and heat-treated foods were evaluated using the chi-square test or Fisher's exact test, as appropriate. Crude prevalence ratios (PRs) with corresponding 95% CIs were calculated to compare detection rates across food categories, using agricultural-derived foods as the reference group. All tests were two-sided, and a p value < 0.05 was considered statistically significant. Statistical analyses were performed using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Overall Detection of Foodborne Pathogens

Between January 2021 and December 2024, a total of 7683 food samples were collected from food-service establishments in Seoul, comprising 1570 non-heated foods and 6113 heat-treated foods (Table 1). Among the non-heated food samples, forty-eight samples were positive for at least one bacterial pathogen; no sample contained more than one pathogen, yielding an overall detection rate of 3.06% (48/1570; 95% CI: 2.31–4.03%). In contrast, heat-treated foods showed a lower detection rate of 1.23% (75/6113; 95% CI: 0.99–1.54%). The difference in detection rates between non-heated and heat-treated foods was statistically significant ($p < 0.001$).

Across all food samples collected during the four-year surveillance period, 123 samples were positive, corresponding to an overall detection rate of 1.60% (95% CI: 1.34–1.91%). The distribution of detected bacterial organisms and their regulatory compliance status is summarized in Figure 1.

Bars represent the number of non-heated food samples in which each bacterial organism was detected. Blue segments indicate detections meeting MFDS microbiological criteria (compliant), and red segments indicate samples classified as non-compliant. For zero-tolerance organisms, detection in 25 g was considered non-compliant. For organisms with quantitative criteria, non-compliance was defined as measured concentrations exceeding MFDS thresholds. Generic *E. coli* is presented as a hygiene indicator organism and is distinguished from pathogenic *E. coli* categories. Total non-heated samples analyzed: $n = 1570$.

Year-specific detection rates for non-heated foods ranged from 2.53% to 3.76%, while those for total foods ranged from 1.24% to 2.14%, with corresponding confidence intervals presented in Table 1.

Table 1. Annual detection and regulatory non-compliance of bacterial organisms in non-heated and heat-treated foods, Seoul, Republic of Korea, 2021–2024. Data are presented as number tested, number detected, number exceeding MFDS criteria, detection rate (%), and 95% confidence intervals (Wilson score method). Detection rates represent unweighted proportions.

Year	Year Distribution of Non-Heated Food Samples	No. of Food Samples	No. of Detected	No. of Exceeding MFDS Criteria	Detection Rate (%)	95% CI (Wilson)
2021	Non-heated food samples	319	12	3	3.76	2.16–6.46
	Year total	1699	32	4	1.88	1.34–2.65
2022	Non-heated food samples	355	12	1	3.38	1.94–1.81
	Year total	1777	38	4	2.14	1.56–2.92
2023	Non-heated food samples	369	10	2	2.53	1.38–4.59
	Year total	2111	27	3	1.28	0.88–1.85
2024	Non-heated food samples	500	14	2	2.80	1.68–4.64
	Year total	2096	26	3	1.24	0.85–1.81
Total	Non-heated food samples	1570	48	8	3.06	2.31–4.03
	4 Years total	7683	123	14	1.60	1.34–1.91

Non-heated foods refer to foods not subjected to heat treatment prior to consumption. Abbreviations: MFDS, Ministry of Food and Drug Safety.

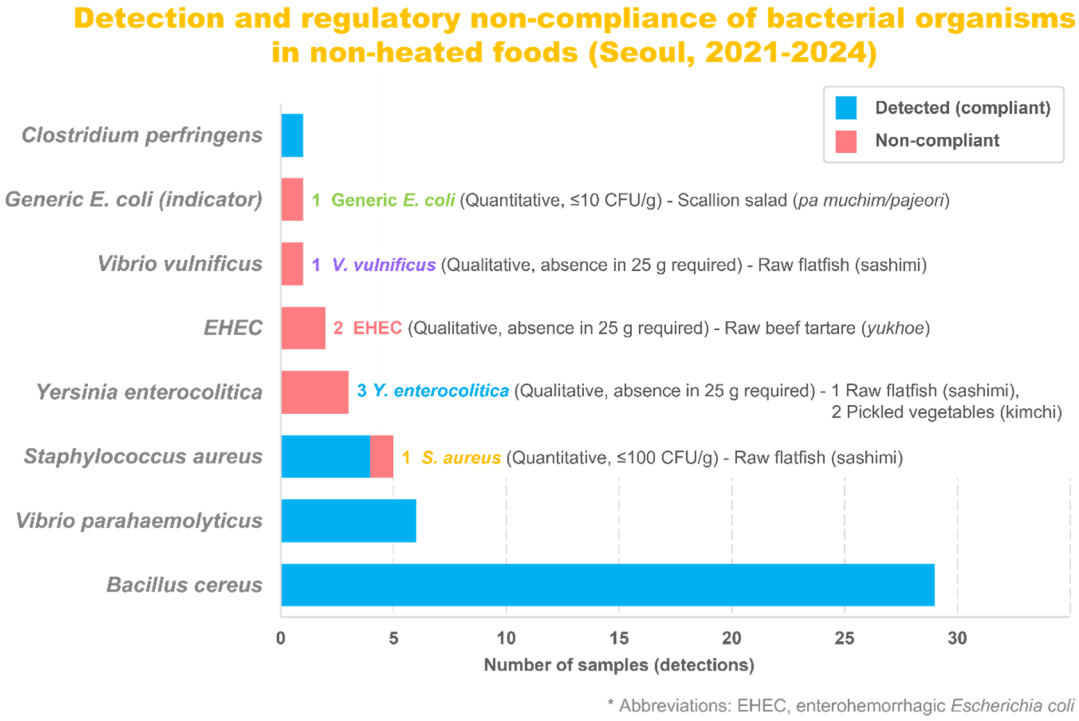

Figure 1. Distribution of detected bacterial organisms and regulatory compliance status in non-heated foods collected in Seoul, 2021–2024.

3.2. Detection by Food Category

Detection rates varied substantially across food categories (Figure 2).

Bars represent detection rates (%) of bacterial pathogens in non-heated foods stratified by food group. Error bars represent 95% confidence intervals (Wilson method). Detection rates were calculated as unweighted proportions (number of positive samples divided by total samples tested). Sample sizes were: agricultural-derived foods (29/1450), fishery-derived foods (14/77), and animal-derived foods (5/43). Agricultural-derived foods were used as the reference category for prevalence ratio estimation in the Results section.

Fishery-derived non-heated foods showed the highest detection rate at 18.18% (14/77; 95% CI: 11.15–28.24%), followed by animal-derived foods at 11.63% (5/43; 95% CI: 5.07–24.48%). In contrast, agricultural-derived foods exhibited a markedly lower detection rate of 2.00% (29/1450; 95% CI: 1.39–2.86%). Overall differences in detection rates among food categories were statistically significant ($p < 0.001$).

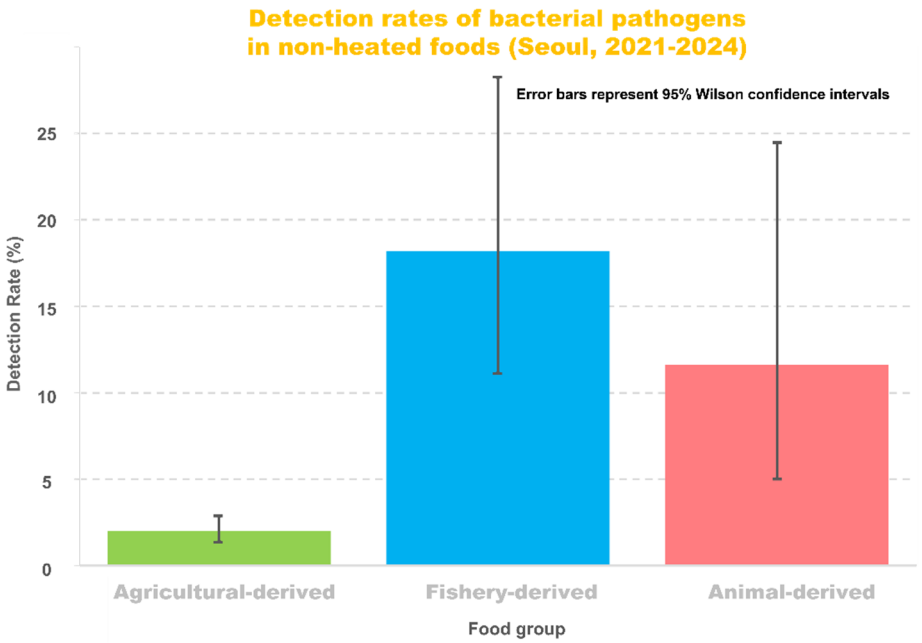

Figure 2. Detection rates of bacterial organisms in non-heated foods by food group. Error bars indicate 95% confidence intervals.

Using agricultural-derived foods as the reference category, the crude prevalence ratio (PR) was 9.09 (95% CI: 4.87–14.83) for fishery-derived foods and 5.81 (95% CI: 2.12–13.97) for animal-derived foods, indicating substantially elevated contamination risks in non-heated foods of animal and fishery origin.

3.3. Non-Compliance with Regulatory Standards

Eight non-heated food samples were determined to be non-compliant according to MFDS microbiological criteria (Supplementary Table S3). Detailed information on the non-compliant samples, including year, food item, and regulatory criterion, is summarized in Table 2, while the complete sample-level dataset is provided in Supplementary Table S1.

The annual distribution of non-compliant samples by bacterial species is presented in Figure 3.

Table 2. Non-compliant non-heated food samples exceeding MFDS microbiological criteria, Seoul, Republic of Korea, 2021–2024. The table summarizes year, pathogen, implicated food item, MFDS microbiological criterion, food-service setting, and number of non-compliant samples. For zero-tolerance organisms, absence in 25 g was required; detection constituted non-compliance. For organisms with quantitative limits, non-compliance was defined as measured concentrations exceeding organism-specific thresholds.

Year	Pathogen	Implicated Food	MFDS Microbiological Limit	Food Service Setting	Total Cases
2021	Enterohaemorrhagic *Escherichia coli*	Raw beef tartare (yukhoe, a Korean raw beef dish)	Absence in 25 g (zero tolerance)	Raw beef specialty restaurant	2
	Yersinia enterocolitica	Pickled vegetables (kimchi)	Absence in 25 g (zero tolerance)	-	1
2022	*Yersinia enterocolitica*	Raw flatfish (sashimi, consumed as sliced raw fish)	Absence in 25 g (zero tolerance)	Sushi restaurant	1
2023	*Vibrio vulnificus*	Raw flatfish (sashimi, consumed as sliced raw fish)	Absence in 25 g (zero tolerance)	Sushi restaurant	1
	Yersinia enterocolitica	Pickled vegetables (kimchi)	Absence in 25 g (zero tolerance)	-	1
2024	*Staphylococcus aureus*	Raw flatfish (sashimi, consumed as sliced raw fish)	≤100 CFU/g	Sushi restaurant	1
	Generic *Escherichia coli*	Scallion salad (pa muchim/pajeori, a Korean seasoned green onion dish)	≤10 CFU/g	Grilled fish restaurant	1

Abbreviations: MFDS, Ministry of Food and Drug Safety.

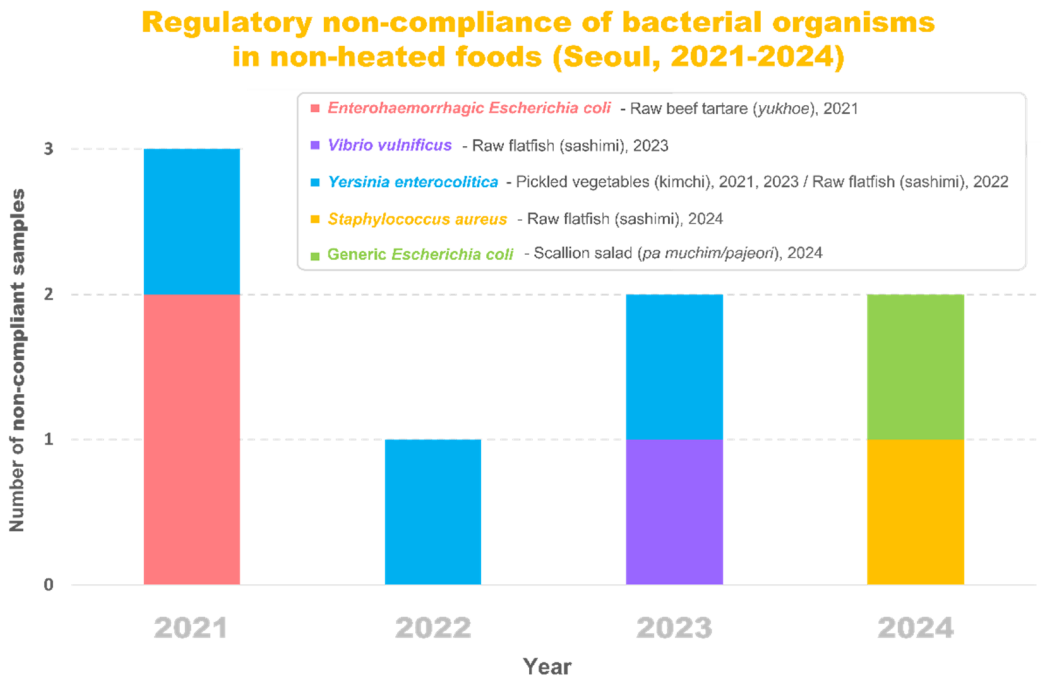

Figure 3. Annual distribution of non-compliant non-heated food samples by bacterial species according to MFDS microbiological criteria, 2021–2024.

Bars represent the number of non-heated food samples classified as non-compliant according to Korean Ministry of Food and Drug Safety (MFDS) microbiological criteria. For organisms subject to zero-tolerance standards, absence in 25 g was required; detection constituted non-compliance. For organisms with quantitative limits, non-compliance was defined as measured concentrations exceeding organism-specific thresholds (≤100 CFU/g for *Staphylococcus aureus*; ≤10 CFU/g for generic *Escherichia coli*). Each sample was treated as an independent analytical unit.

Non-compliance included detection of zero-tolerance pathogens (enterohemorrhagic *Escherichia coli*, *Yersinia enterocolitica*, and *Vibrio vulnificus*) and quantitative exceedances of *Staphylococcus aureus* and generic *Escherichia coli*. Regulatory criteria corresponding to these five organisms are detailed in Supplementary Table S3.

4. Discussion

This multi-year municipal surveillance study provides epidemiologically meaningful estimates of bacterial detection in non-heated foods and identifies food categories associated with elevated contamination risks. The overall detection rate in non-heated foods exceeded that observed in heat-treated foods, reinforcing the recognized vulnerability of foods served without a final lethal heat treatment [2,4]. The interpretation of non-compliance strictly followed MFDS-defined microbiological criteria, with qualitative (presence/absence) and quantitative (CFU/g) standards clearly distinguished to avoid misclassification.

Consistent with previous surveillance studies, animal- and fishery-derived foods exhibited substantially higher contamination rates than agricultural products [5,6]. In particular, fishery-derived foods exhibited the highest detection rate within the sampled population, aligning with reports that highlight the susceptibility of raw or minimally processed seafood to *Vibrio* species and other marine-associated bacteria [11]. Although the number of animal-derived samples was relatively small, the elevated prevalence ratio and wide confidence interval suggest a potentially meaningful risk that warrants continued monitoring. Sensitivity analyses excluding samples exceeding quantitative limits yielded similar prevalence patterns, supporting the robustness of the observed category-specific differences.

Bacillus cereus was the most frequently detected pathogen, reflecting its ubiquitous presence in the environment and its ability to survive under diverse storage conditions [3]. The detection of regulatory non-compliance events involving EHEC, *Yersinia enterocolitica*, and *Vibrio vulnificus* underscores the public health importance of strict hygiene management in non-heated foods, particularly those of animal and fishery origin.

No enteric viruses were detected during the study period. This finding may reflect factors such as sample type, assay sensitivity, and the episodic nature of viral contamination [8]. Importantly, the absence of detection in

routine surveillance does not necessarily indicate the absence of risk, emphasizing the need for continued methodological refinement and targeted viral monitoring.

The higher detection rates observed during the early years of the study period may reflect temporal variation in food-service practices as well as seasonal influences on food consumption and microbial growth [6]. Taken together, these findings support the prioritization of non-heated animal- and fishery-derived foods in risk-based surveillance programs and emphasize the importance of cold-chain control, prevention of cross-contamination, and continuous training for food handlers in urban food-service environments.

Municipal surveillance observations (data not shown) have identified instances in which EHEC was detected in vegetable components and condiments rather than in thermally processed primary ingredients, suggesting that non-heated produce may function as downstream vehicles of contamination even when core ingredients undergo adequate heat treatment. Similarly, surveillance findings (data not shown) indicate that *Vibrio* contamination may be associated not only with raw seafood itself but also with handling practices and holding environments in food-service settings, underscoring the importance of environmental hygiene and cross-contamination control. Although *Salmonella* was not detected in non-heated foods in the present dataset, routine surveillance observations (data not shown) have documented contamination events in upstream food components such as eggshell surfaces, highlighting the need for integrated farm-to-service hygiene management.

5. Limitations

This study has several limitations. First, as a routine surveillance–based analysis, the sampling strategy was determined by municipal inspection plans rather than by a probabilistic design, which may limit the generalizability of prevalence estimates beyond the inspected establishments. Second, although statistically significant differences were observed between food categories, the relatively small number of animal- and fishery-derived samples resulted in wide confidence intervals, and residual confounding factors such as establishment size, seasonality, and specific food handling practices were not controlled for. Because potential clustering by establishment was not accounted for, the reported confidence intervals may slightly underestimate variability. Third, the absence of detected enteric viruses should be interpreted cautiously, as viral contamination may be episodic and influenced by sampling frequency and assay sensitivity. Nevertheless, the large sample size, multi-year study period, and standardized laboratory procedures strengthen the internal validity of the findings and provide robust epidemiological evidence to inform risk-based food safety management in urban food-service settings.

6. Conclusions

This multi-year municipal surveillance study demonstrates that non-heated foods served in food-service establishments exhibited higher bacterial detection rates than heat-treated foods within the monitored population. Although overall detection rates were relatively low, non-heated animal- and fishery-derived foods showed disproportionately higher detection frequencies, and regulatory non-compliance events involving high-risk pathogens such as EHEC and *Vibrio vulnificus* were identified.

These findings support prioritizing non-heated foods, particularly those of animal and fishery origin, within risk-based surveillance and hygiene management strategies in densely populated urban environments. Strengthened cold-chain control, prevention of cross-contamination during preparation, and seasonally informed monitoring may help reduce foodborne disease risks in food-service settings.

Conducted within a routine municipal surveillance framework, this study provides practical public health evidence to inform targeted food safety policies and inspection priorities. Continued integration of surveillance data with outbreak investigations and risk assessment activities will be important for enhancing early detection and prevention of foodborne diseases in urban food-service environments.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/260402135811701 6/IJFSC-26010086-SI-FC.pdf. Table S1: Individual non-heated food samples classified as non-compliant according to MFDS microbiological criteria, Seoul, Republic of Korea, 2021–2024; Table S2: Analytical procedures for detection and enumeration of target bacterial organisms according to the MFDS Food Code (2024 edition); Table S3: MFDS microbiological criteria, measurement basis, and definitions of non-compliance for organisms classified as non-compliant in this study.

Author Contributions

Y.E.K.: conceptualization, methodology, formal analysis, writing—original draft preparation, writing—review and editing; Y.-H.C.: data curation, investigation; J.L.: methodology, validation; S.P.: investigation; B.K.: investigation; J.Y.: data curation; H.K.: data curation; O.-H.K.: data curation; S.J.: supervision. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Ethical review and approval were waived for this study because it was conducted as part of a legally mandated public health surveillance program and did not involve human subjects or identifiable personal data.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available upon reasonable request from the corresponding author. The data are not publicly available due to institutional and regulatory restrictions related to public health surveillance data.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI-assisted technologies were used in the preparation of this manuscript.

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