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Supplementary Materials

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

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Table S1. Individual non-heated food samples classified as non-compliant according to MFDS microbiological criteria, Seoul, Republic of Korea, 2021–2024. The table presents individual 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; presence 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 *E. coli*). Measured level indicates qualitative presence/absence or quantitative concentration (CFU/g) as determined by routine laboratory testing.

Sample ID	Year	Food Category	Food Item	Pathogen	MFDS Standard	Measured Level
SB-03-11-G1	2021	Agricultural-derived	Pickled vegetables (kimchi)	*Yersinia enterocolitica*	Absence in 25 g (zero tolerance)	Presence (qualitative)
SP-08-24-01	2021	Animal-derived	Raw beef tartare (yukhoe)	EHEC	Absence in 25 g (zero tolerance)	Presence (qualitative)
SC-12-03-02	2021	Animal-derived	Raw beef tartare (yukhoe)	EHEC	Absence in 25 g (zero tolerance)	Presence (qualitative)
DB-03-22-01	2022	Fishery-derived	Raw flatfish (sashimi)	*Yersinia enterocolitica*	Absence in 25 g (zero tolerance)	Presence (qualitative)
SD-03-30-01	2021	Agricultural-derived	Pickled vegetables (kimchi)	*Yersinia enterocolitica*	Absence in 25 g (zero tolerance)	Presence (qualitative)
SP-09-21-06	2023	Fishery-derived	Raw flatfish (sashimi)	*Vibrio vulnificus*	Absence in 25 g (zero tolerance)	Presence (qualitative)
GN-03-25-83	2024	Fishery-derived	Raw flatfish (sashimi)	*Staphylococcus aureus*	≤100 CFU/g	200 CFU/g (quantitative)
GS-06-19-04	2024	Agricultural-derived	Scallion salad (pa muchim/pajeori)	Generic *Escherichia coli*	≤10 CFU/g	90 CFU/g (quantitative)

Abbreviations: MFDS, Ministry of Food and Drug Safety; EHEC, enterohemorrhagic *Escherichia coli*.

Table S2. Analytical procedures for detection and enumeration of target bacterial organisms according to the MFDS Food Code (2024 edition). Analytical procedures were performed in accordance with the Korean Ministry of Food and Drug Safety (MFDS) Food Code (2024 edition). The table summarizes sample preparation, enrichment or plating conditions, confirmation methods, and reporting units applied in this study. Quantitative results are expressed as colony-forming units per gram (CFU/g), calculated using the confirmed colony count multiplied by the dilution factor unless otherwise specified. Qualitative analyses were interpreted as presence/absence in 25 g of sample. For zero-tolerance organisms, non-compliance was defined as detection in any test portion.

Organism	Food Code	Test Type	Sample prep.	Culture/Detection Conditions	Confirmation	Result
Bacillus cereus	8.4.18.2	Quantitative only (non-heated foods)	25 g + 225 mL diluent; Homogenized, 10-fold serial dilution	0.3/0.4/0.3 mL on MYP agar; 30°C, 24 h	Lecithinase reaction and biochemical confirmation	CFU/g (confirmed count × dilution factor)
Vibrio parahaemolyticus	8.4.13.2	Quantitative only (non-heated foods)	25 g + 225 mL diluent; Homogenized, 10-fold serial dilution	0.3/0.4/0.3 mL on TCBS; 35–37°C, 18–24 h	≥5 colonies confirmed (sucrose(-)/biochemical)	CFU/g (confirmed count × dilution factor)
Staphylococcus aureus	8.4.12.2	Quantitative only (non-heated foods)	25 g + 225 mL diluent; Homogenized, 10-fold serial dilution	0.3/0.4/0.3 mL on BPA; 35–37°C, 48 ± 3 h	≥5 colonies confirmed (coagulase/biochemical)	CFU/g (confirmed count × dilution factor)
Escherichia coli (generic)	8.4.8.2	Quantitative only (non-heated foods)	25 g + 225 mL diluent; 1 mL plated on duplicate dry films	35 ± 1°C, 24–48 h incubation	Colony color criteria; MPN if necessary	CFU/g (mean count × dilution factor)
Clostridium perfringens	8.4.14.2	Quantitative only (non-heated foods)	25 g + 225 mL diluent; 10-fold serial dilution 1 mL plated in duplicate	TSC agar (43-45°C, pour + overlay) → Anaerobic, 35–37°C, 24 ± 2 h	≤150 black colonies enumerated and biochemical confirmation	CFU/g (confirmed count × dilution factor)
Yersinia enterocolitica	8.4.17	Qualitative	25 g + 225 mL PSBB; 10 mL transferred to 90 mL ITC broth	25°C, 48 h → 0.1 mL KOH treatment → MacConkey/CIN plating	Motility at 25°C (+), 37°C(−); Urea(+); Citrate(-)	Presence /absence in 25 g
EHEC	8.4.16	Qualitative	25 g + 225 mL mTSB; Homogenized	35-37°C, 24 h → TC-SMAC/BCIG plating	PCR for *stx1, stx2* genes + serological confirmation	Presence /absence in 25 g
Vibrio vulnificus	8.4.27	Qualitative	25 g + 225 mL APW; Homogenized	35-37°C, 18–24 h → TCBS/mCPC plating	LIM, oxidase(+), LDC(+), Indole(+); NaCl tolerance	Presence /absence in 25 g
Salmonella spp.	8.4.11	Qualitative	25 g + 225 mL BPW; Homogenized	Pre-enrichment 35-37°C, 18–24 h → RV/TT → XLD/BG Sulfa	Biochemical and serological confirmation	Presence /absence in 25 g
Listeria monocytogenes	8.4.15	Qualitative	25 g + 225 mL LEB; Homogenized	30°C, 48 h (Secondary enrichment) → Oxford/ALOA plating	Biochemical Identification (PI-PLC, CAMP test)	Presence /absence in 25 g
Campylobacter jejuni/coli	8.4.19	Qualitative	25 g + 100 mL Bolton broth; Homogenized	35-37°C, 4-5 h (microaerophilic) → 42 ± 1°C, 44 ± 4 h → mCCDA plating	Biochemical Identification (catalase/oxidase)	Presence /absence in 25 g

Abbreviations: ALOA, Agar Listeria according to Ottaviani & Agosti; APW, Alkaline Peptone Water; BCIG, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide; BG Sulfa, Brilliant Green Sulfa agar; BPA, Baird-Parker Agar; BPW, Buffered Peptone Water; CAMP, Christie-Atkins-Munch-Petersen; CIN, Cefsulodin-Irgasan-Novobiocin agar; EHEC, Enterohemorrhagic *Escherichia coli*; ITC, Irgasan-Ticarcillin-Chlorate broth; LDC, lysine decarboxylase; LDC, Lysine decarboxylase; LEB, Listeria Enrichment Broth; LIM, Lysine Iron Medium; mCCDA, Modified Charcoal Cefoperazone Deoxycholate Agar; mCPC, Modified Cellobiose-Polymyxin-Colistin agar; MPN, Most Probable Number; mTSB, modified Tryptic Soy Broth; MYP, Mannitol-Egg Yolk-Polymyxin agar; PCR, Polymerase Chain Reaction; PI-PLC, Phosphatidylinositol-specific phospholipase C; PSBB, phosphate-buffered saline with bovine serum; RV, Rappaport-Vassiliadis broth; TCBS, Thiosulfate-Citrate-Bile salts-Sucrose agar; TSC, Tryptose Sulfite Cycloserine agar; TT, Tetrathionate broth; XLD, Xylose-Lysine-Deoxycholate agar.

Table S3. MFDS microbiological criteria, measurement basis, and definitions of non-compliance for organisms classified as non-compliant in this study. This table presents the MFDS microbiological criteria applicable to the five organisms classified as non-compliant in this study. For zero-tolerance pathogens, non-compliance is defined as detection in any test portion (presence in 25 g). For organisms with quantitative standards, non-compliance is defined as measured values exceeding the established CFU/g threshold. Criteria for organisms not resulting in non-compliance are defined in the MFDS Food Code but are not reproduced here.

Organism	MFDS Microbiological Criterion	Measurement Basis	Definition of Non-Compliance
EHEC	Zero tolerance (absence in 25 g)	Presence/absence	Detection in any test portion
Yersinia enterocolitica	Zero tolerance (absence in 25 g)	Presence/absence	Detection in any test portion
Vibrio vulnificus	Zero tolerance (absence in 25 g)	Presence/absence	Detection in any test portion
Staphylococcus aureus	≤100 CFU/g	Quantitative (CFU/g)	Measured value >100 CFU/g
Generic *Escherichia coli* (hygiene indicator)	≤10 CFU/g (depending on food category)	Quantitative (CFU/g)	Measured value exceeding category-specific threshold

Abbreviations: MFDS, Ministry of Food and Drug Safety; EHEC, enterohemorrhagic *Escherichia coli*.