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Food-borne outbreak of *Listeria monocytogenes* in school students in Seoul, Korea

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ABSTRACT

In September 2018, an outbreak of Listeriosis cases in Korea was traced to food involved, using retrospective cohort studies and PFGE analyses. This study aimed to describe the first *L.monocytogenes* outbreak identified in Korea. We confirmed the presence of Serovar (4C) and virulence genes, and evaluated the genetic correlation between isolates by restriction digestion patterns of *Apa*I and *Asc*I. Based on the epidemiological association, it is presumed that the seasoned crab meat with bean sprouts are contaminated by cross contamination during the bean sprouts washing (relative risk was 1.24; p-value: 0.0021 and they possessed virulence genes. Therefore, active laboratory surveillance is necessary to recognize the risk of *L. monocytogenes* in Korea.

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1. Introduction

Human Listeriosis is a significant public health concern and a leading cause of hospitalization and death due to foodborne illness (1). *Listeria monocytogenes* is a facultative intracellular pathogen that causes a rare, yet severe, human illness; typical symptoms include septicemia, abortions, meningitis, and encephalitis (2). *L. monocytogenes* has also been involved in several febrile gastroenteritis outbreaks (3).

Listeriosis can be a serious disease with >95% hospitalization and an approximate 20% mortality; that case–fatality rate may increase in groups at highest risk (4,5), who are the pregnant women, neonates, elderly, immunocompromised individuals and adults with malignancy (6). *L. monocytogenes* outbreaks in the US have recently been linked to consumption of packaged salad, soft cheese and cantaloupes, and the pathogen caused 292 deaths or fetal losses from 2009 to 2011, with a mortality rate of approximately 21% (7,8). However, to date, *L. monocytogenes* has not been reported as a cause of large outbreak in Korea.

In this study, for the first time, we report the presence of L. monocytogenes due to food poisoning and the epidemiologic characteristics of *L. monocytogenes* isolated from Seoul, Korea.

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2. Materials and Methods

2.1. Subjects and specimen collection

It was reported that 64 people reported gastrointestinal diseases of A middle and B high school students who use the same cafeteria at south east Public Health Center in Seoul, and were infected with *L. monocytogenes* on September 5th, 2018. Illnesses started on dates ranging from September 4, 2018 to November 12, 2018. Case surveys were distributed to collect student and teacher demographics, clinical characteristics, and patterns of food consumption. Fecal samples were taken from the patients who had more than 2 occurrences of fever, abdominal pain, diarrhea, vomiting among those who consumed food provided in the school cafeteria. The cafeteria and kitchens of the schools were tested for environmental exposure to pathogens. Processed food, unprocessed food, knife, dishcloth, cutting board, and drinking water were collected and inspected by Seoul Metropolitan Government Research Institute of Public Health and Environment Consumption analysis was performed on 2,096 exposed individuals who responded to the epidemiological study conducted via surveys and phone interviews.

2.2. Survey investigation

According to a standard questionnaire administered by a health institution, there were 244 students, 34 food handlers, 14 faculty members and 2 pregnant school teachers. This was a retrospective cohort study on exposed subjects. For statistical analysis, Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA) and Epi Info 3.2 (Centers for Disease Control and Prevention, Atlanta, GA, USA) were used (Table 1). The P-value for statistical significance is defined as $p < 0.05$ in this study. The response and research activities that take place during the large scale outbreak are legally delegated by the government controlled infectious disease control system, so approval and prior consent of the institutional review committee were not required. In relative risk analysis of all food items served between 31 August and 4 September, it was conducted to confirm the relationship between food and disease.

2.3. Isolation of *L. monocytogenes*

Fecal samples were collected during the investigation to monitor pathogens routinely isolated from patients with food-borne diarrhea, including 10 bacterial pathogens (pathogenic *E. coli, Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus, Campylobacter* spp., *Staphylococcus aureus, Clostridium perfringens, Listeria monocytogenes, Yersinia enterocolitica*, and *Bacillus cereus*) and 5 viruses (rotavirus, norovirus, adenovirus, sapovirus, and astrovirus) as described previously (9,10). *L. monocytogenes* was also identified using Bruker Biotyper MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) (11).

2.4.Serotyping

Serotype analysis of L. monocytogenes isolates was performed by slide aggregation assay using commercially prepared antiserum (*Listeria antiserum* Seiken kit; DenkaSeikenCo, Tokyo, Japan) according to the manufacturer's instructions.

2.5. Preparation of genomic DNA

Genomic DNA is extracted according to the manufacturer's instructions using AccuPrep®Genomic DNA Extraction Kit (Bioneer, Korea).

2.6. Identification by the multiplex-PCR of virulence-associated genes

All primers used for specific PCR amplification of the entire coding sequence of the virulence-related gene are reported in Table 2. PCR was performed in a PCR 9600 thermal cycler (Perkin-Elmer Corporation). The reaction conditions consisted of template DNA denaturation (94°C for 3 min), 35 cycles of amplification (each cycle was denaturation at 94°C for 1 min, annealing at 60°C for 2 min, and elongation at 72°C 1 min), and visualization under UV.

Table 1. Attack rates and RRs of gastroenteritis among 2,096 persons having meals

Date	Meal	Exposed		Attack rate (%)	Non-Exposed		Attack rate (%)	RR(95% CI)
		No. of cased	Total		No. of cased	Total		
Lunch Sep 3, 2018	Multi-grain Rice	1359	509	37.5	264	76	28.8	1.30 (1.06, 1.59)
	Soybean paste stew with Snails	1108	416	37.5	394	151	38.3	0.98 (0.85,1.13)
	Stir-fried Pork	1322	497	37.6	284	85	29.9	1.26 (1.04,1.52)
	Seasoned Crab meat with Bean sprouts	908	392	43.2	532	185	34.8	1.24 (1.08,1.43)
	Kimchi	891	331	37.1	537	228	42.5	0.88 (0.77,1.00)
	Green apple Ade	1321	497	37.6	288	84	29.2	1.29 (1.06,1.56)
Dinner Sep 3, 2018	Fried rice with Hurigake	323	132	40.9	949	453	47.7	0.86 (0.74,0.99)
	Spicy Cold Chewy Noodles	308	123	39.9	963	461	47.9	0.83 (0.72,0.97)
	Clear Soybean Soup	299	123	41.1	968	462	47.7	0.86 (0.74,1.00)
	Fried chicken Skewer	327	132	40.4	948	454	47.9	0.84 (0.72,0.98)
	Green Fudding Salad	268	107	39.9	994	478	48.1	0.83 (0.71,0.97)
	Sliced Radish Kimchi	246	98	39.8	1016	483	47.5	0.83 (0.71,0.99)

RR, relative risk; CI, confidence interval.

Table 2. Primer pairs used for amplification of virulence genes and 16s rRNA in *Listeria* isolates

Primers	Sequences (5'→3')	Product size(bp)
inlA	F CCT AGC AGG TCT AAC CGC AC R TCG CTA ATT TGG TTA TGC CC	255
inlB	F AAA GCA CGA TTT CAT GGG AG R ACA TAG CCT TGT TTG GTC GG	146
actA	F GAC GAA AAT CCC GAA GTG AA R CTA GCG AAG GTG CTG TTT CC	385
hlyA	F GCA TCT GCA TTC AAT AAA GA R TGT CAC TGC ATC TCC GTG GT	174
plcA	F CGA GCA AAA CAG CAA CGA TA R CCG CGG ACA TCT TTT AAT GT	129
plcB	F GGG AAA TTT GAC ACA GCG TT R ATT TTC GGG TAG TCC GCT TT	261
16S rRNA	F CAG CAG CCG CGG TAA TAC R CTC CAT AAA GGT GAC CCT	938

PFGE

PulseNet is the national subtyping network of public health and food regulatory agency laboratories coordinated by KCDC. PFGE was performed according to the PulseNet standardized protocol (12), KCDC PulseNet manages a national database of these DNA fingerprints to identify possible outbreaks (Figure. 1).

3. Results

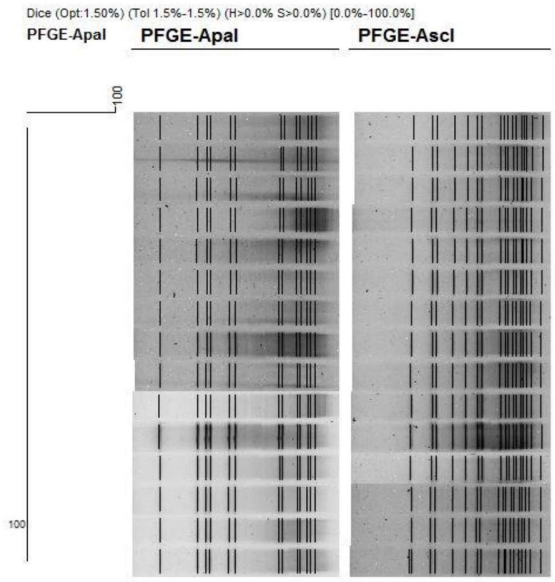
The numbers of food samples taken; 37 preserved food, 4 drinking water, 2 cookware, 19 refrigerator handle, 1 bean sprout, 1 food water, and 4 estimated contaminated foodstuffs.

During the outbreak period, we identified 64 potential cases of *L. monocytogenes*. Dates of onset of symptoms ranged from 3 to 6 September 2018 (Figure 2). Attack rate was 31.2% (653 of 2,096). Approximately 2,096 students visited the school cafeteria and 294 cases were found to have prevalence rate of 14.0%.

Symptoms included abdominal pain (37.7%), diarrhea (31.7%), chill (31.1%), fever (27.7%), nausea (23.0%), headache (11.5%), and vomiting (6.4%).
Among the 10 bacterial and 5 viral pathogens tested, which are commonly involved in the foodborne diarrhea, 64 *L. monocytogenes* strains were isolated which were serotyped as serotype 4C.

Of the food samples collected in school, L. monocytogenes were isolated from Seasoned Crab meat with bean sprouts (lunch), Spicy Cold Chewy Noodles (dinner), and Green Fudding Salad (dinner) on Sep. 3, 2018 (Table 1) (Figure 2). In relative risk analysis of all food items served between 3 and 7 September, seasoned crab meat with bean sprouts was significantly associated with occurrence of disease (relative risk 1.24; 95% CI: 1.08, 1.43) (Table 1).

Six different virulence-related genes and PCR products of 16s rRNA (Table 2) were obtained from DNA from all Listeria strains considered in this study, and the isolates were 16S rRNA and seven toxicity-related genes (hlyA, plcA, plcB, inlA, inlB, actA and prfA), suggesting potentially pathogenic (Figure. 3). The PFGE (ApaI and AscI) types of all isolates were presented in the dendrogram analysis of the PFGE profile, indicating that 64 *L. monocytogenes* isolates belong to the same PFGE profile, including LITA16.001 for ApaI and LITA12.001 for AscI, respectably (Figure. 1).



Year	ApaI pattern	AscI pattern	Note
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Stool
2018	LITA16.001	LITA12.001	Food
2018	LITA16.001	LITA12.001	Food
2018	LITA16.001	LITA12.001	Food

Figure 1. Dendrogram of *Apa*I and *Asc*I PFGE profiles of *L. momocytogenes* isolates. The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean method. Degrees of similarity (% values) are shown.

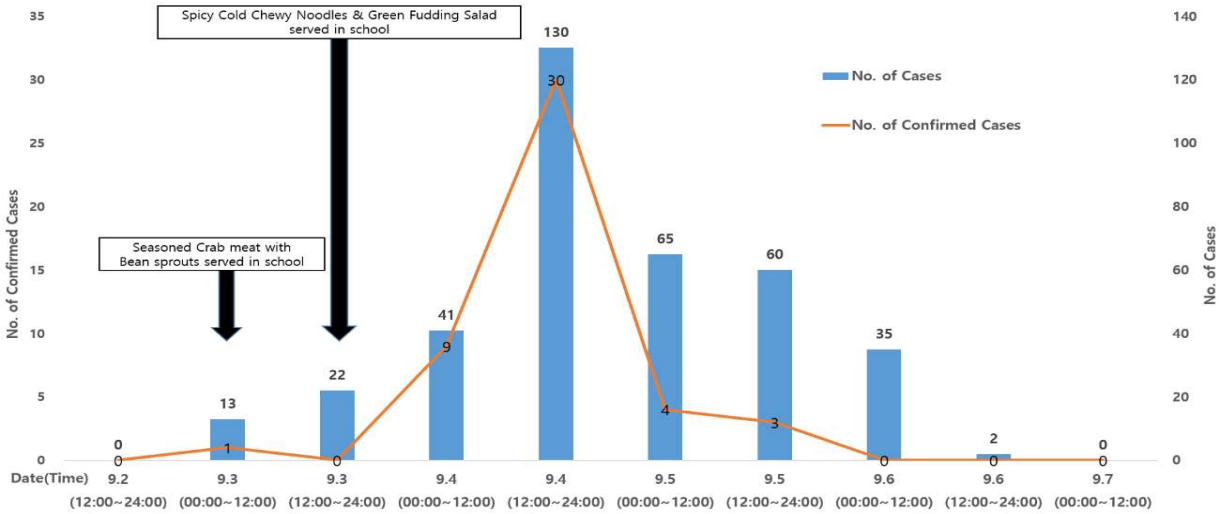


Figure 2. Cases of *L. momocytogenes* associated with consumption of Seasoned Crab meat with Bean sprouts, Spicy Cold Chewy Noodles, Green Fudding Salad in A and B schools, Republic of Korea in 2018 (n=368), according to date of illness onset.

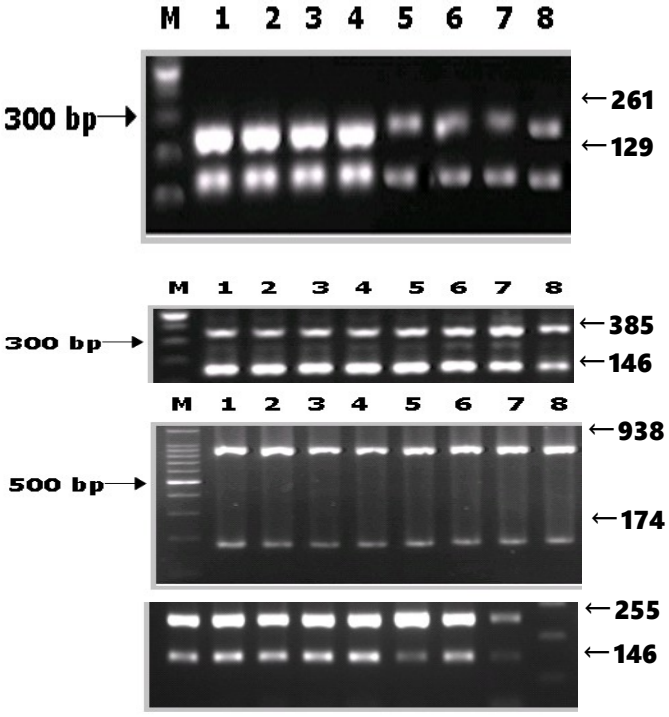


Figure 3. Multiplex PCR results on agarose gel electrophoresis for the identification of *L. monocytogenes*. In the multiplex PCR plcA (129bp), plcB (261), inlB (146 bp), actA (385 bp), hlyA (174 bp), 16S rRNA (938 bp), inlA (255 bp) were used as primers.); 1-8:*L.monocytogenes* isolates.

4. Discussion

This report describes the outbreak of the first food poisoning *L. monocytogenes* identified in Korea. Eating seasoned crab meat with bean sprouts was significantly associated with illness by using retrospective cohort studies (relative risk was 1.24; p-value: 0.0021) and PFGE analyses. The outbreak affected 64 persons from two schools (middle and high) in Seoul and was associated with the consumption of contaminated seasoned crab meat with bean sprouts.

No deaths have been reported. Seoul Metropolitan Government Research Institute of Public Health and Environment has identified 64 ill people infected and food with the same DNA fingerprint of *L. monocytogenes*. Epidemiologic evidence from the KCDC PFGE result indicated that seasoned crab meat with bean sprouts is a likely source of the outbreak. To the best of our knowledge, this study is the first report that outbreak of Listeriosis cases in Korea is traced to food involved, using retrospective cohort studies and PFGE analyses.

Although *L. monocytogenes* causes relatively few cases of human infectious disease in Korea and around the world, it is still a major problem in public health because it exists extensively in many animals, foods, raw materials, and the environment (12-15). There are many factors contributing to an increased risk of Listeriosis, among them three major factors may contribute to an increased incidence of Listeriosis; increased susceptibility of the population, increased exposure to *L. monocytogenes* and, finally, improved diagnosing and case surveillance (16). We investigated retrospective cohort studies by interviewing everyone who ate at the school cafeteria. The risk exposure period was estimated based on the prevalence curve, the food intake ability, the detection bacteria, and the incubation period. It is estimated that patients were the first exposed from the school lunch on Sep. 4, 2018, and then second exposure from dinner. It is estimated that the minimum latency period is 7 h, the longest latency period is 240 days, and the average latency period is 5 days. Previous studies have shown that Listeriosis can have a long incubation period (median 11 days, range 0–70 days) between exposure and symptom onset (17,18).

Among the provided meals, listeria was detected in the seasoned crab meat with bean sprouts (lunch), spicy cold chewy noodles (dinner), and green fudding salad (dinner) on Sep. 3, 2018, and all of the menus were provided with raw vegetables, among which seasoned crab meat with bean sprouts was statistically most significant. Considering that the same pathogen was detected in the foods (spicy cold chewy noodles, and green fudding salad) of dinner menu, it is reasonable to assume that cross contamination was caused by the vegetables washed together during the bean sprouts washing. Consumption of contaminated food made under unsanitary conditions will be an important cause of Listeriosis outbreaks in Korea. PFGE of the *Listeria* strains isolated from food samples collected in school were found to be the same sequences of Listeria strains isolated from 64 people who became ill from 3 through 6 Sep. 2018.

Since the onset of Listeriosis takes weeks to days, it can be very difficult to identify the source of the infection timely. It is very important to quickly identify and report through epidemiological surveys to find the possible source of the contamination. Thus, it is not surprising that many sporadic occurrences and some cases have not been addressed to locate contaminated food (4). Although, limited information is available about the seasoned crab meat with bean sprouts that ill people consumed, the PFGE findings, together with the seasoned crab meat with bean sprouts consumption history of all patients and inspection findings at the school kitchen, suggest that these illnesses could be related to the seasoned crab meat with bean sprouts.

Studies have shown that ready-to-eat contaminated foods such as cheese, milk, and beef transmit *L. monocytogenes* to humans (19-21) and novel vehicles (i.e., sprouts, taco/nacho salad) were associated with outbreaks in this study (22,23). These results indicate the need of implementing hygienic rules in the cleaning and cooking process of vegetables to ensure microbiological safety and to improve shelf life. In addition, due to the development of food storage technology, it has been confirmed that refrigeration temperature growth bacteria (24,25), *Listeria*, can be detected in almost all foods that are consumed without

heating. This suggests that Korea is no longer a safe zone for the outbreak of Listeria.

5. Conclusions

This study aimed to describe the first *L.monocytogenes* outbreak identified in Korea. Serovars (4C) and presence of virulence genes were determined. Based on the epidemiological association, it is presumed that the seasoned crab meat with bean sprouts are contaminated by cross contamination during the bean sprouts washing (relative risk was 1.24; p-value: 0.0021), and they possessed virulence genes. In order to avoid listeria outbreaks in the future, we believe that hygiene education should be addressed for listeria high risk groups (the elderly, immunocompromised individuals, and pregnant women). To the best of our knowledge, this study is the first report a large scale outbreak of listeria in Korea. This discovery highlights the importance of strong hygiene and L. monocytogenes monitoring programs for school cafeteria.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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