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Research Note

Salmonella Prevalence and Total Microbial and Spore Populations in Spices Imported to Japan

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ABSTRACT

A total of 259 samples of 40 types of spices were tested for *Salmonella* prevalence and total microbial and spore populations. *Salmonella enterica* serotypes Weltevreden and Senftenberg were isolated from a black- and red-pepper sample, respectively. Because *Salmonella* was not detected by the most-probable-number method, it indicated that at least one cell of the microorganism was present in 25 g of sample. The mean aerobic bacterial count was greater than 5.39 log CFU/g in turmeric, garam masala, curry powder, and paprika. The mean bacterial spore counts were greater than 4.33 log CFU/g in turmeric and curry powder. The mean aerobic bacterial count in the two *Salmonella*-isolated samples was 6.93 log CFU/g. These results indicate that spices can be a source of contamination in the products where they are used as ingredients, and methods to reduce the microbial load in spices should be used.

Salmonella is a major cause of foodborne illness worldwide. Various types of food, including eggs *(1, 14),* chicken *(3),* fish *(22),* and vegetables *(15)* have been implicated in foodborne salmonellosis outbreaks. *Salmonella* is known to have a high tolerance to stress such as desiccation and has actually been reported to have been isolated from dried food *(7, 25).* Spices, one type of dried food, have been reported to show *Salmonella* contamination *(24),* and sometimes the international food trade between countries is impacted due to *Salmonella* contamination *(5).* Spices are usually used as ingredient in preparing various food products. In some foods such as curry, several types and large volumes of spices are used and the finished products are traditionally subjected to a heat treatment during preparation. However, spices such as pepper are often used in food preparation in small volumes where they are added to food as a final flavoring or for taste after cooking or just before eating. If these spices are contaminated with *Salmonella,* the consumption of such products can result in a foodborne illness. Some outbreaks of salmonellosis have been epidemiologically linked to the consumption of foods seasoned with black or white pepper *(4)* and paprika-powdered potato chips *(8).* The food material which causes foodborne infections is unknown in many cases. This might be due to difficulties to detect pathogens in food because the contamination level is generally very low and there is a higher level of competitive microflora in such foods. In addition, the food materials might not be analyzed for pathogens because of the small amounts used in foods. Spices can be a cause of foodborne illness if they are used in foods after the foods are heat processed.

In this study, the microbiological quality of commercial spices imported from overseas to Japan was evaluated along with the prevalence of *Salmonella* in spices.

MATERIALS AND METHODS

Sample. A total of 259 samples of 40 types of commercial spices imported into Japan were purchased in retail shops in Tokyo (Table 1). The samples were kept at room temperature and tested within 3 days. The spices originated from various, but mostly from Asian, countries such as China, India, and Malaysia.

Detection of *Salmonella*. After the outside of the sample bag was disinfected with 80% ethanol, the bag was unsealed using sterilized scissors. A portion (25 g) of samples was transferred to a stomacher bag or a beaker using a sterilized spoon. Samples other than clove, cinnamon, and oregano were added to 225 ml of tryptic soy broth (TSB; Difco, Sparks, Md.) in a stomacher bag. For clove, cinnamon, and oregano, 25 g of the sample was added to 2,500 ml of TSB in a beaker *(23).* After incubation at room temperature for 1 h, the pH was adjusted to 6.8. The samples were incubated at 35°C for 24 h and portions (1.0 and 0.1 ml) of each culture were added to 10 ml of tetrathionate (TT) broth (Oxoid, Basingstoke, Hampshire, UK) and Rappaport-Vassiliadis (RV) broth (Oxoid), respectively. After incubation at 42°C for 24 h, the cultures were streaked onto two plates each of XLD agar (Oxoid) and CHROMagar *Salmonella* (CHROMagar, Paris, France), directly or after immunomagnetic separation (IMS) with Dynabeads anti-*Salmonella* (Dynal, Oslo, Norway), according to

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TABLE 1. *Detection of* Salmonella *in commercial spices*

Type of spice	No. of samples tested	(country of origin)	No. (%) of *Salmonella*-positive samples
Red pepper	59	(21: China, 5: Korea, 5: Thailand, 2: Sri Lanka, 2: Uzbekistan, 1: India, 1: China and Korea, 22: Unknown)	1 (1.7)[a]
Black pepper	42	(6: Malaysia, 5: China, 5: India, 2: Micronesia, 1: Vietnam, 1: United States, 22: Unknown)	1 (2.4)[b]
White pepper	23	(6: China, 2: Malaysia, 2: Micronesia, 1: Thailand, 1: Indonesia, 11: Unknown)	0
Cumin	16	(3: China, 2: India, 1: Pakistan, 10: Unknown)	0
Curry powder	14	(5: India, 1: Malaysia, 1: Sri Lanka, 7: Unknown)	0
Coriander	12	(1: Morocco, 1: Thailand, 1: India, 1: Morocco and Canada, 8: Unknown)	0
Fennel	9	(4: India, 2: China, 3: Unknown)	0
Paprika	7	(1: Korea, 6: Unknown)	0
Cinnamon	7	(2: China, 1: Vietnam, 1: Indonesia, 3: Unknown)	0
Japanese spice	7	(4: China, 1: Japan, 2: Unknown)	0
Garlic	6	(2: China, 1: United States, 3: Unknown)	0
Garam masala	5	(1: India, 1: Pakistan, 3: Unknown)	0
Clove	5	(2: China, 3: Unknown)	0
Anise	4	(4: Unknown)	0
Star anise	4	(4: China)	0
Caraway	3	(3: Unknown)	0
Turmeric	3	(1: United States, 2: Unknown)	0
Fenugreek	3	(2: Pakistan, 1: Unknown)	0
Allspice	3	(3: Unknown)	0
Green pepper	3	(1: India, 1: Thailand, 1: Unknown)	0
Chinese five spice	2	(1: China, 1: Unknown)	0
Nutmeg	2	(2: Unknown)	0
Bay leaves	2	(1: China, 1: United States and Turkey)	0
Artemisia	2	(1: China, 1: Japan)	0
Long pepper	1	(1: India)	0
Curry leaf	1	(1: Unknown)	0
Ajowan	1	(1: India)	0
Green coriander	1	(1: India)	0
Mustard	1	(1: Canada)	0
Basil	1	(1: United States)	0
Sage	1	(1: France and Turkey)	0
Dill weed	1	(1: United States)	0
Oregano	1	(1: Turkey)	0
Parsley	1	(1: United States)	0
Celery	1	(1: India)	0
Mandarin	1	(1: Unknown)	0
Spice mixture A[c]	1	(1: China)	0
Spice mixture B[d]	1	(1: China)	0
Spice mixture C[e]	1	(1: Thailand)	0
Spice mixture D[f]	1	(1: Unknown)	0
Total	259		2 (0.8)

[a] The country of origin was unknown. *Salmonella enterica*; the serotype was Senftenberg.
[b] The country of origin was Thailand. *Salmonella enterica*; the serotype was Weltevreden.
[c] Spice mixture A: cumin, red pepper, and others.
[d] Spice mixture B: Japanese spice and others.
[e] Spice mixture C: spices for Thai food, but the components were unknown.
[f] Spice mixture D: the components were unknown.

the manufacturer's instructions. After incubation at 35°C for 24 h, a portion of the suspected colonies was tested for agglutination with a *Salmonella* antibody kit (Unipath, Oxoid) and confirmed using the biochemical characteristics on triple sugar iron agar (Eiken Chemical, Tokyo, Japan) and lysine indole motility agar (Eiken Chemical). Confirmed isolates were further serotyped for agglutination against *Salmonella* O and H antigens (Denkaseiken, Tokyo, Japan).

Furthermore, samples in which *Salmonella* was detected were tested to quantify the contamination levels using a three-tube most-probable-number (MPN) method. In the same manner as described above, homogenates of samples in 225 ml of TSB in a stomacher bag were prepared. Each 10, 1, and 0.1 ml of the homogenates was transferred to 10 ml of TSB and cultured at 35°C for 24 h. The culture was incubated in TT and RV broth and then plated onto agar plates to isolate *Salmonella.*

TABLE 2. *Aerobic and spore-forming bacteria counts in commercial spices*

	No. of samples	Aerobic bacteria (log CFU/g)				Spore-forming bacteria (log CFU/g)				No. (%) of samples with a spore-forming bacteria count of >1,000 CFU/g
		Mean	SD	Maximum	Minimum	Mean	SD	Maximum	Minimum	
Red pepper	59	3.99	1.98	7.05	<1.00	1.91	1.51	5.61	<1.00	13 (22.0)
Black pepper	42	4.29	2.41	6.68	<1.00	2.57	1.96	6.76	<1.00	16 (38.1)
White pepper	23	3.88	1.51	5.85	<1.00	1.81	1.21	4.53	<1.00	5 (21.7)
Cumin	16	4.05	1.05	5.99	1.85	2.26	1.30	4.92	<1.00	4 (25.0)
Curry powder	14	5.83	0.93	6.96	3.23	4.33	1.64	6.30	1.18	10 (71.4)
Coriander	12	5.28	0.59	6.05	4.37	4.04	0.72	4.96	2.73	11 (91.7)
Fennel	9	3.67	0.45	4.43	3.06	1.50	0.56	2.30	<1.00	0 (0)
Paprika	7	5.39	2.00	6.43	<1.00	1.97	1.74	4.66	<1.00	2 (28.6)
Cinnamon	7	3.74	0.64	4.69	3.00	1.16	0.36	1.78	<1.00	0 (0)
Japanese spice	7	3.83	2.35	6.20	<1.00	1.53	0.64	2.65	<1.00	0 (0)
Garlic	6	3.16	1.16	4.49	1.17	1.11	0.39	1.90	<1.00	0 (0)
Garam masala	5	6.02	0.63	6.43	4.91	3.45	1.69	5.07	1.17	3 (60.0)
Clove	5	1.82	1.19	3.30	<1.00	1.06	0.23	1.48	<1.00	0 (0)
Anise	4	3.20	2.35	6.20	<1.00	1.95	1.43	3.98	<1.00	1 (25.0)
Star anise	4	3.04	0.83	4.23	2.30	<1.00	0.00	<1.00	<1.00	0 (0)
Caraway	3	3.26	0.68	4.00	2.66	1.19	0.36	1.60	<1.00	0 (0)
Turmeric	3	6.89	1.70	8.85	5.86	4.91	1.83	6.38	2.86	2 (66.7)
Fenugreek	3	5.02	1.86	6.16	2.87	3.00	1.73	4.02	1.00	2 (66.7)
Allspice	3	5.25	0.18	5.44	5.08	1.72	1.33	3.26	<1.00	1 (33.3)
Green pepper	3	2.89	3.35	6.75	<1.00	<1.00	0.00	<1.00	<1.00	0 (0)

Aerobic and spore-forming bacteria count. Samples (5 g) were added to 95 ml of phosphate-buffered saline (PBS, pH 7.0) and incubated at room temperature for 1 h. For the spore-forming bacterial count, 20 ml of the sample solution was transferred to a glass tube and heated at 100°C for 10 min. The heated solution for the spore-forming bacteria count and nontreated solution for the aerobic bacteria count were diluted 10^1 to 10^4-fold in PBS. A portion (1 ml) of the dilutions of each sample in PBS and 20 ml of plate count agar (Nissui, Tokyo, Japan) were poured, incubated at 35°C for 48 h, and counted *(12).*

PCR assay. To detect the *invA* gene in the enrichment culture of spice samples, 1 ml of each sample cultured in TSB was transferred to a microtube and the DNA was extracted using a DNA extraction kit (Mag Extractor-Genome, Toyobo, Osaka, Japan). In addition, cultures of *Salmonella* isolates, incubated in TSB at 35°C for 24 h, were used for DNA extraction as described for enrichment culture spice samples.

PCR to target *invA* was performed as follows: the PCR reaction mixture (50 μl) contained 0.4 μM each of primer set (i.e., *invA*139 and 141) *(10),* 0.2 mM each of the four deoxynucleoside triphosphates (dNTP mixture, Takara Bio Inc., Ohtsu, Japan), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 mM EDTA, 0.1 mM dithiothreitol, 0.05% Tween 20, 0.05% Nonidet P-40, 5% glycerol, 0.5 U of *Taq* polymerase (Takara *Ex Taq,* Takara), template DNA solution (2.5 μl), and distilled water (34.75 μl). The reaction was performed at 94°C for 1 min for denaturing, 55°C for 1 min for annealing, and 72°C for 1 min for extension using a thermal cycler (ABI9700, Applied BioSystems, Foster City, Calif.). After 35 cycles, samples were finally heated to 72°C for 10 min. PCR products were applied to electrophoresis on 3% agarose gel. After staining with ethidium bromide, the size of the PCR product (378 bp) was compared to that of a *Salmonella* Enteritidis strain as a positive control using UV lighting.

Data analysis. The six spices with 12 or more samples were tested to determine correlation coefficients (r^2 value) between aerobic bacteria and spore-forming bacteria. Correlation coefficients were calculated by Microsoft Excel 2001 (Microsoft, Redmond, Wash.).

RESULTS

Based on the results of the PCR assay and culture method, *Salmonella* was detected in 1 of 59 samples of red pepper and 1 of 42 samples of black pepper (Table 1). Because *Salmonella* was not detected by the MPN method, the contamination level of *Salmonella* was <30 MPN/100 g in both samples, but at least one cell of the microorganism was present in 25 g of sample or 0.04 cell per g. *Salmonella* isolates from the black- and red-pepper samples were *Salmonella enterica* serotypes Weltevreden and Senftenberg, respectively.

Mean aerobic bacterial counts were 6.89, 6.02, 5.83, 5.39, 5.33 log CFU/g in turmeric, garam masala, curry powder, paprika, and coriander, respectively (Table 2). The mean counts in clove and star anise were 1.82 and 3.04 log CFU/g, respectively. Although the mean aerobic bacterial counts in black- and red-pepper samples were 4.29 and 3.99, respectively, those of the black-pepper and red-pepper samples in which *Salmonella* was detected were 6.93 and 6.88 log CFU/g, respectively.

The mean spore-forming bacteria counts were 4.91 and 4.33 log CFU/g in turmeric and curry powder, respectively; the mean of many of the spice samples was <2.0 log CFU/g (Table 2). Although the mean spore-forming bacterial count of red pepper was 1.91 log CFU/g, that of red pepper in which *Salmonella* was detected was 5.61 log CFU/g.

The correlation between aerobic bacterial count and spore-forming bacteria count for six types of spices (red pepper, black pepper, white pepper, cumin, curry powder,

and coriander) was analyzed. There was generally no apparent relationship between the aerobic bacterial count and spore-forming bacteria count. However, the r^2 values were 0.85 and 0.80 in black pepper and coriander samples, respectively. The samples of black pepper seemed to separate into two groups: one group contained more than 5.5 log CFU/g of aerobic bacteria and 3.0 log CFU/g of spore-forming bacteria, and the other group contained less than 4.2 log CFU/g of aerobic bacteria and 3.0 log CFU/g of spore-forming bacteria.

DISCUSSION

In this study, *Salmonella* was found in one sample of red pepper (1.7%) and one sample of black pepper (2.4%). Pafumi *(17)* reported that *Salmonella* was isolated from black and white peppercorns and fenugreek seed at a relatively high incidence level (8.2, 1.5, and 7.1%, respectively). D'Aoust *(4)* described the importance of spices as internationally traded food as reservoirs of *Salmonella* since the prevalence of *Salmonella* in pepper from Brazil and several Asiatic countries was 6.7 to 13.8%. Satchell et al. *(21)* also described *Salmonella* contamination in black pepper from Indonesia and Brazil. Rampersad et al. *(19)* reported that *Salmonella* was isolated from spices at a rate of 1% (2 of 200 samples) in Thailand. Although the samples in the present study consisted of several types of spices originating from various countries, *Salmonella* was isolated from only two types of spices (red and black pepper). It is possible that further evaluation of the spices from the same commercial brand in which the pathogen was detected could have revealed a greater prevalence of *Salmonella* from these sources.

Salmonella contamination in both positive samples was estimated at 0.04 CFU/g. However, it seems that the samples were contaminated with *Salmonella* at a very low concentration and the microorganism was likely irregularly distributed in the spices. A greater sample volume and more replicate analyses are necessary to determine precise contamination levels in spices.

Salmonella-positive red- and black-pepper samples contained greater populations of aerobic and spore-forming bacteria than the non–*Salmonella*-positive samples of each type of spice. The presence of *Salmonella* in a sample may be related to a high frequency and level of bacterial contamination from the environment or animals. The methods of harvesting and sanitization might be important to reduce the contamination with *Salmonella.*

We considered the possibility that the grinding process of spice corns increased the bacterial population in spice powder due to bacterial contamination. To confirm this, we tested two samples of black peppercorns after being ground into powder using a sanitized mill. The bacterial populations of powder were similar to those of the peppercorns before grinding (data not shown). Thus, the form of spices does not affect the bacterial count. Rather, bacteria might contaminate spices during the grinding process in the factory.

Salmonella enterica serotypes Weltevreden and Senftenberg isolated from the spices in this study were frequently isolated from meat and shrimp in Vietnam *(18)* and samples from patients with diarrhea in some countries such as Singapore *(16),* the Philippines *(11),* or Thailand *(20).* In addition, the U.S. Food and Drug Administration reported that *S. enterica* serotype Weltevreden was the most frequently isolated serotype from seafood imported into the United States between 1990 to 1998 and *S. enterica* serotype Senftenberg was the next *(6). S. enterica* serotypes Weltevreden and Senftenberg are rarely isolated from patients in Japan and other serotypes such as Thompson or Newport are more common *(6, 13).* Imported foods, including seafood and spices, might be important in foodborne illness in Japan as in the United States.

In this study, we demonstrated that spices can be contaminated with *Salmonella,* although at a low concentration. Some treatments to destroy pathogens such as fumigation *(2)* and storage of food containing spices at low temperature *(9)* are important to control *Salmonella* infection.

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