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Prevalence, serotype diversity, and antimicrobial resistance of *Salmonella* in imported shipments of spice offered for entry to the United States, FY2007–FY2009

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ABSTRACT

In response to increased concerns about spice safety, the U.S. FDA initiated research to characterize the prevalence of *Salmonella* in imported spices. Shipments of imported spices offered for entry to the United Sates were sampled during the fiscal years 2007–2009. The mean shipment prevalence for *Salmonella* was 0.066 (95% CI 0.057–0.076). A wide diversity of *Salmonella* serotypes was isolated from spices; no single serotype constituted more than 7% of the isolates. A small percentage of spice shipments were contaminated with antimicrobial-resistant *Salmonella* strains (8.3%). Trends in shipment prevalence for *Salmonella* associated with spice properties, extent of processing, and export country, were examined. A larger proportion of shipments of spices derived from fruit/seeds or leaves of plants were contaminated than those derived from the bark/flower of spice plants. *Salmonella* prevalence was larger for shipments of ground/cracked capsicum and coriander than for shipments of their whole spice counterparts. No difference in prevalence was observed between shipments of spice blends and non-blended spices. Some shipments reported to have been subjected to a pathogen reduction treatment prior to being offered for U.S. entry were found contaminated. Statistical differences in *Salmonella* shipment prevalence were also identified on the basis of export country.

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1. Introduction

In 2006, FDA reported that *Salmonella* contamination of spices was the cause of 95% of the U.S. food recalls associated with spices over the period 1980–2000 (Vij et al., 2006). Between 2007 and 2010, three large-scale salmonellosis outbreaks in the United States were attributed to consumption of *Salmonella*-contaminated spices/seasonings (Sotir et al., 2009; CDC, 2010; Higa, 2011). Since that time, FDA established the Reportable Food Registry (RFR), an early warning system that enables industry and public health officials to report hazards in foods before the food reaches the consumer (USFDA, 2012a,b). In its first two years of reporting (Sept. 2009–Sept 2011; USFDA, 2012c–d), "spices and seasonings" led nearly all human food categories in total number of RFR primary entries (ranked 2nd in years 1 and 2) and number of primary entries associated with *Salmonella* (ranked 1st in year 1 and 2nd in year 2). The present study is a part of a larger effort by the United States Food and Drug Administration (FDA) to conduct spice safety research to assess the salmonellosis public health risk posed by spice consumption in the U.S. and to assist the agency in identifying options to mitigate the risk (USFDA, 2010).

The U.S. is one of the largest importers of spices, on the basis of both volume and value (Buzzanell et al., 1995), with more than 80% of the total U.S. spice supply provided by imports (USDA/ERS, 2011). The present study examines FDA *Salmonella* surveillance sampling and testing results for a variety of imported spices offered for entry to the U.S. over a three year period. In addition to determining an average *Salmonella* prevalence value for spices sampled, this study examines whether *Salmonella* prevalence is strongly dependent on the type of spice, extent of processing, and country of export. Subtype and antimicrobial resistance information provide insights into the diversity of *Salmonella* found in spices. This is the first comprehensive study of *Salmonella* contamination of spices imported into the United States, updating a very limited study in 1987–8 (Satchell et al., 1989) and complementing the examination of recall events from 1980 to 2000 (Vij et al., 2006).

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2. Materials and methods

2.1. Sample collection

Imported spice and other FDA-regulated food samples were collected from shipments offered for entry to the U.S. and analyzed by FDA during the period October 1, 2006 and September 30, 2009 or fiscal years 2007–2009 (FY2007–FY2009). The samples were collected as part of FDA's annual field work plan which defines resource allocation including product categories to be sampled. Selection of spice/other food shipments for examination was based on a number of factors including the inherent risk of the product, general surveillance activities described in the FDA work plan, FDA work performance goals and/or congressional work performance goals. All data examined in this study were drawn from "surveillance sampling activities", as described above, as opposed to compliance activities related to public health emergencies, such as foodborne illness outbreak investigations. Further, sampling from shipments already associated with import alerts for *Salmonella* and follow-up sampling related to food safety emergencies were not included in this study.

Imported shipments of spices included in this study were restricted to dried spices, which include foods classified as spices when offered for import plus dried capsicums and sesame seeds classified as vegetables/vegetable products and edible seeds, respectively. Extracts, liquid spices/seasonings, imitation flavors and salt were excluded from the present analysis. Imported shipments of all other FDA-regulated foods collected during this time period, except color additives (a category which can include cosmetic as well as food additives), were included in this study. A full description of FDA product code classifications is available online (USFDA, 2012e).

Sampling plans used in this study followed that described in FDA's Bacteriological Analytical Manual (BAM) (Andrews and Hammack, 2003). The number of subsamples collected from a shipment depended on the classification of that food as a FDA Food Category I, II, or III food (Andrews and Hammack, 2003). Spices were generally sampled as a Category II food. For both spices and other foods, subsamples, each comprised of approximately 160 g, were collected randomly from each shipment. Typically, each subsample was collected from a different sack/container of food in the shipment. Subsamples were sent to U.S. Food and Drug Administration (FDA) laboratories for analysis.

2.2. Sample preparation, Salmonella screening, isolation and confirmation

Composite samples of spices or other FDA-regulated foods were prepared according to the protocol described in FDA's BAM (Andrews and Hammack, 2003). Generally, the 60, 30, or 15 subsamples collected for Category I, II, or III foods, respectively, were divided into four, two or one group of fifteen. Twenty-five gram analytical units of product from each of the fifteen subsamples were combined to form a 375 g composite sample. Each composite spice sample was screened for the presence of *Salmonella* using one of the following methods: AOAC's Official Methods (OMA) methods 2004.03 and 996.08. Other foods were examined with these methods or other AOAC official methods (AOAC International, 2011). In some cases, e.g., when insufficient sample was available or when FDA recommended a different sampling plan for a particular food (such as exotic meat), detection protocols deviated from those described above.

FDA's BAM recommends the use of modified analysis procedures for several spices (Andrews and Hammack, 2003). Briefly, K_2SO_3 is added to the trypticase soy broth when analyzing dehydrated onion or garlic for *Salmonella* and dilutions of 1/100 are used when analyzing allspice, cinnamon/cassia, and oregano for *Salmonella*. For cloves, a dilution of 1/1000 is used for *Salmonella* analysis. For the analyses involving dilutions, the entire 375 g composite is homogenized in the diluted broth but only a fraction of the homogenate is examined for *Salmonella*: 1/10 of the homogenate for samples of allspice, cinnamon/cassia, or oregano and 1/100 of the homogenate for samples of cloves (Hammack, 2012). As a result, tests for these four spices are expected to have reduced detection sensitivities.

Salmonella was isolated from each of the composite samples testing positive using the procedures described in Chapter 5 of FDA's BAM (Andrews and Hammack, 2011). Presumptive-positive *Salmonella* isolates were confirmed with OMA methods 978.24 or 991.13.

2.2.1. Salmonella speciation and antimicrobial susceptibility testing

Salmonella serotypes were determined or characterized to the extent possible for all isolates recovered from contaminated spice samples examined in this study (Ewing, 1986). Bacterial cultures were prepared and submitted as directed and specified in FDA's BAM (Andrews and Hammack, 2003).

Salmonella isolates from spices were assayed for susceptibility to antimicrobials with antimicrobial minimum inhibitory concentrations (MIC) determined via the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2012), except for the MIC value for streptomycin (for which there is no CLSI guidelines), where the U.S. National Antimicrobial Resistance Monitoring System (NARMS) interpretive criteria (USFDA, 2012f) was used. All *Salmonella* isolates were tested for susceptibility to the following antimicrobials: amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, sulfisoxazole, and trimethoprim/sulfamethoxazole, tetracycline, and streptomycin.

2.3. Statistical methods

Standard statistical tests were employed in the analysis to determine confidence intervals and determine significance of observed differences. Confidence intervals (CI) reported are exact (Clopper–Pearson) binomial confidence intervals (SAS, 2012). Differences among pairs of data were assessed for significance using the Fisher exact test whereas differences in prevalence values between multiple spice groups (e.g., different spice varieties) were evaluated for significance with the chi-square test for multiple proportions (NIST/SEMATECH, 2012). In cases where the null hypothesis was rejected by the chi-square test for multiple proportions, pairwise comparisons were made using the Marascuillo procedure (NIST/SEMATECH, 2012).

3. Results and discussion

3.1. Observed prevalence of Salmonella in imported spice shipments offered for entry to the U.S.

Results of the three-year surveillance sampling study are presented in Table 1. Since some spices examined in the study were sampled using a protocol different from the standard FDA Category II Food protocol described in Section 2.1, a Fisher exact test was applied to determine whether there was a statistical difference in *Salmonella* prevalence for these shipments as compared with those sampled using the Category II food protocol; no difference was

Table 1
Frequency and prevalence of *Salmonella*-contaminated imported spice shipments and other imported FDA-regulated food shipments offered for entry to the U.S., FY2007–FY2009.

Spice/food	# positive	*N*	*Salmonella* shipment prevalence	Confidence interval (95%)[a]
All imported spices[b]	187	2844	0.066	0.057–0.076
All other imported FDA-regulated foods[b]	600	17,508	0.034	0.032–0.037
Categories of spices[c]				
Fruit[d]	92	1465	0.063	0.051–0.080
Root[d]	15	202	0.074	0.042–0.12
Leaf[d]	18	160	0.11	0.068–0.17
Bark/flower[d]	1	66	0.02	0.0–0.1
Spices subjected to different processes				
Spices subjected to a pathogen reduction treatment[e]	4	137	0.03	0.008–0.07
Spices not treated/not known if treated[e]	183	2707	0.068	0.058–0.078
Spice blend[f]	43	790	0.054	0.040–0.073
Spice not-blend[f]	141	1999	0.071	0.060–0.083
Ground/cracked spice	131	1658	0.079	0.066–0.093
Whole spice	51	884	0.058	0.043–0.075
Specific spices[g]				
Capsicum[h]	35	492	0.071	0.050–0.098
Cinnamon/clove/nutmeg	1	73	0.01	0.00–0.07
Coriander	16	110	0.15	0.085–0.23
Cumin	11	138	0.080	0.040–0.14
Curry powder	17	195	0.087	0.052–0.14
Fennel/fenugreek/mustard	3	112	0.027	0.01–0.08
Oregano/basil	10	82	0.12	0.060–0.21
Pepper, black	13	291	0.045	0.024–0.075
Pepper, white	1	87	0.01	0.00–0.06
Sesame seed	20	177	0.11	0.070–0.17
Turmeric	8	118	0.07	0.03–0.1
Spices/spices & Seasonings, NEC[i]	32	685	0.047	0.032–0.065
All other spices	20	284	0.070	0.044–0.11

[a] 95% exact (Clopper–Pearson) confidence limit (SAS, 2012).
[b] All shipments of imported FDA-regulated spices or other imported foods that were sampled during the study period.
[c] Categorizations derived from product code (USFDA, 2012e) and description. When description was insufficient to categorize, the sample was not included.
[d] Categorization of spice shipment based on the part of the plant from which it is derived. Seeds and fruits are both categorized as "fruit".
[e] Spice shipment classified as "commercially sterile", "heat treated" or "irradiated" and those in which the product description identified treatment (e.g., "treated with steam" or "treated with ethylene oxide") are categorized as "Treated Spices". All other spices are categorized as "Not Treated/Not Known if treated".
[f] The category "Spice Blend" includes shipments of spice mixtures while "Spice Not Blend" includes shipments of a single type of spice.
[g] Different forms of spices with the same name, such as dried coriander leaves and seeds, are grouped together.
[h] Capsicum includes paprika as well as hot and other sweet dried capsicum peppers.
[i] Shipments of spices "not elsewhere classified" (NEC) in the product code (USFDA, 2012e) are assigned to "Spices, NEC", "Spices & Seasonings, NEC", or "Mixed Spices and Seasonings, NEC".

found. Therefore results for spices are compared in Table 1 without regard to sampling protocol employed.

Salmonella prevalence in imported spice shipments offered for entry to the U.S. during FY2007–FY2009 was 0.066 (95% CI 0.057–0.076). This value does not differ statistically from the value determined by FDA thirty years ago for examination of a set of 31 imported spice shipments offered for entry to the U.S.: prevalence = 0.06 (95% CI 0.008–0.2), Satchell et al. (1989). These two studies are the only studies to examine the prevalence of *Salmonella* contamination of spices in the U.S. and help to place the frequency of U.S. recalls (Vij et al., 2006) and RFR entries (USFDA, 2012c–d) associated with *Salmonella*-contaminated spice into context.

The *Salmonella* shipment prevalence determined in the present study can be compared with values determined for spices in other countries, Table 2. All of the values listed in Table 2 are from surveillance studies; most samples were collected from retail establishments. Further, it is likely that a majority of spices examined in Australia, Federal Republic of Yugoslavia, Ireland, Japan, and the United Kingdom were imported because these countries are not major producers of the spices examined. A majority of the studies summarized in Table 2 found observed *Salmonella* prevalence values in the range of zero to one percent and nearly all of the prevalence values in Table 2 are statistically smaller than the value determined in the present study (Fisher exact test, $p < 0.05$); only exceptions include the retail study in Brazil that found a value of 0.056 (95% CI 0.030–0.094; Moreira et al., 2009), and two studies that examined a very small number of samples (16 samples of sesame seeds collected at retail in Germany (Brockmann et al., 2004) and 25 batch samples of spices from pre-retail establishments in Ireland (Food Safety Authority of Ireland, 2005)). The screening test protocols in all of the studies in Table 2 examined a smaller mass of spice than that used in the present study which means that the test sensitivity was also smaller. Therefore, it is likely that at least some of the observed difference between the smaller *Salmonella* prevalence values reported in Table 2 and the value reported in the present study arises from test sensitivity differences. Assuming the distribution of *Salmonella* among and within shipments/spice packages is similar to that observed by FDA among capsicum shipments and sesame seeds shipments offered for entry to the U.S. (zero-inflated gamma-Poisson distribution with parameters determined in Van Doren et al., submitted for publication), we would expect the observed *Salmonella* prevalence to be 2–3 times smaller when 25 g is examined as compared with the 750 g used in the present study. A much larger reduction in observed *Salmonella* prevalence would be expected if the *Salmonella* level distribution mean was smaller or the range among shipments was narrower than that found in FDA's 2010 study (Van Doren et al., submitted for publication). The smaller prevalence values reported in the different countries and settings may also

Table 2
Summary of other scientific surveillance studies measuring the prevalence of *Salmonella* in spices, 2000–2012.

Country[a]	Sample collection point	Sample size (g)[b]	*N*	Prevalence	95% CI[c]	Spices sampled[d]	Spices containing *Salmonella*[e]	Reference
Australia	Retail	125*	217	0	0.00–0.01	Caraway, chili, cloves, coriander, cumin, fennel, fenugreek, ginger, mustard, nutmeg, sumac, turmeric, Chinese five spice mix, garam masala, other spice mixes		Department of Health, State Government of Victoria, Australia (2010)
Australia	Import	25	Not reported	0.005; 0.049[f]		Peppercorn; paprika	Peppercorn; paprika	Department of Health, State Government of Victoria, Australia (2010), Food Standards Australia New Zealand (2001)
Brazil	Retail	25	233	0.056	0.030–0.094	Bay, basil, black pepper, cinnamon, clove, cumin, dehydrated green onion, oregano, parsley	Black pepper, cumin	Moreira et al. (2009)
Egypt	Retail	25	297[g]	0	0.00–0.01 Minus 0.00–0.01	Geranium, basil, marjoram, peppermint, spearmint, jews mallow, dill, celery, parsley, cumin, caraway, anise, fennel, coriander, dill, black pepper, chamomile, karkade, saffron		Abou Donia (2008)
Federal Republic of Yugoslavia[h]	Retail	25	101	0	0.0–0.03	Bay, basil, black pepper, capsicum, caraway, cinnamon, clove, coriander, curry, dill, ginger, mustard, nutmeg, oregano, rosemary, sesame, thyme, white pepper		Stankovic et al. (2006)
Germany	Retail	25	16	0.1	0.02–0.4	Sesame seed	Sesame seed	Brockmann et al. (2004)
India	Retail	25	154	0.01	0.002–0.05	Allspice, aniseed, asafetida, bay (tejpat), bishop's weed, black cumin, black pepper, caraway, cardamom, celery seed (ajmud), chili, cinnamon, clove, coriander, cumin, fenugreek, garlic, ginger, mustard, poppy, turmeric	Ginger, poppy seed	Banerjee and Sakar (2003)
Ireland	Primarily pre-retail[i]	125*	25	0	0.0–0.1	Capsicum, curcuma (including turmeric), ginger, nutmeg, other spices and herbs		Food Safety Authority of Ireland (2005)
Ireland	Primarily retail	25	647	0.0093	0.003–0.02	Capsicum, curcuma (including turmeric), ginger, nutmeg, piper spp. (e.g., black and white pepper), other spices and herbs	Chili pepper & chili powder, curry, sesame seeds, turmeric[j]	
Japan	Retail	25	259	0.008	0.0009–0.03	Allspice, ajowan, anise, artemisia, capsicum, basil, bay leaves, black pepper, capsicum, caraway, celery, Chinese five spice, cinnamon, clove, coriander, cumin, curry powder, curry leaf, dill weed, fennel, fenugreek, garlic, garam masala, mandarin, mustard, nutmeg, oregano, paprika, parsley, sage, star anise, turmeric, white pepper, other dried peppers, other spice mixtures	Black pepper, red pepper	Hara-Kudo et al. (2006)
Mexico	Retail	3	304	0[k]	0.00–0.01	Bay, cumin, garlic, pepper, oregano		Garcia et al. (2001)
Turkey	Retail	25	75	0	0.00–0.04	Allspice, black pepper, cinnamon, cumin, red pepper		Beki and Ulukanli (2008)
Turkey	Spice producers & retail	25	170	0	0.00–0.02	Black pepper, capsicum, cumin, peppermint, thyme		Kahraman and Ozmen (2009)
Turkey	Retail	25	420	0.029	0.015–0.049	Allspice, black pepper, capsicum, coriander, cumin, ginger, white pepper	Allspice, black pepper, coriander, cumin, ginger, red pepper	Hampikyan et al. (2009)
Turkey	Retail	25	65	0	0.00–0.05	Basil, mint, thyme		Ulukanli and Karadag (2010)
United Kingdom	Retail	25	1031[l]	0.01	0.0074–0.023	Alfalfa, poppy, sesame	Alfalfa, sesame seed	Willis et al. (2009); Willis (2012)
United Kingdom	Retail	135*	2833	0.011	0.0074–0.015	Aniseed, allspice, basil, bay, black pepper, capsicum, cinnamon, coltsfoot, coriander, cumin, dill, fennel, fenugreek, garam masala, ginger, lemongrass, mace, mustard, nutmeg, oregano, parsley, saffron, sage, tarragon, thyme, turmeric, white pepper, other piper spp. (e.g., green, red, mixed), other spices and spice mixes[m]	Allspice, black pepper, cayenne, chili, cinnamon, coriander, cumin, curry, fennel, fenugreek, garam masala, mint, okra, sage, turmeric[m]	Sagoo et al. (2009), Little (2011)
United Kingdom	Manufacturing & packing	135*	132	0.01	0.002–0.05			

United Kingdom	Retail	25	386	0.003	0.001–0.01	Spice mixes (not specified)	Spice mix (not specified)	Little et al. (2003)
Multiple countries	Spice producer	25	79	0	0.00–0.04	Saffron		Cosano et al. (2009)

[a] Country were sample was collected.
[b] Total mass examined by *Salmonella* screening test. Star (*) indicates studies tested five sub-samples per spice sample; total mass examined is listed (i.e., five times sub-sample mass).
[c] 95% exact (Clopper–Pearson) confidence limit (SAS, 2012).
[d] Spices sampled list combines different forms of the same kind of spice under one name (e.g., ground and whole caraway seeds are listed as caraway) and combines related species under one name (e.g., cayenne, chili, paprika, and "red pepper" are listed as capsicum). See reference for more detailed list.
[e] Spices containing *Salmonella* list reports spice name as noted in the reference.
[f] Spice-specific prevalence values for peppercorns (0.005) and paprika (0.049).
[g] Does not include tea samples.
[h] Currently the State Union of Serbia and Montenegro.
[i] Majority of samples from importers/distributors, producers/blenders, packers/wholesalers or food manufactures/preparers (establishments using large amount of spice).
[j] Four of six samples testing positive for *Salmonella* were from retail; one turmeric sample was collected from import/production/wholesaler and the curry powder sample was collected from an establishment that uses large amounts of spices for food production.
[k] Samples examined for the presence of *Salmonella* Typhi.
[l] Only includes seed samples (sesame, poppy, and alfalfa).
[m] Sagoo et al. (2009) reported spice types from all sample collection points together.

reflect real differences in prevalence either arising from a difference in the microbiological quality of the raw/imported spices examined or differences resulting from the application of one or more processes. Process treatments such as ethylene oxide, propylene oxide, steam treatment or irradiation are commonly applied to spices to reduce the risk of microbial contamination (American Spice Trade Association, 2011). Some insight into this latter hypothesis is provided in Section 3.1.2, where *Salmonella* shipment contamination prevalence determined in this study are compared on the basis of applied processes. A study examining *Salmonella* prevalence in retail spice samples in the U.S. could provide a direct measure of the efficacy of risk management practices in reducing the prevalence of *Salmonella* contamination post-import.

During the three year study period, spice shipments offered for entry to the U.S. were 1.9 times more likely to be found contaminated than shipments of all other FDA-regulated foods offered for U.S. entry combined (relative risk (RR), 95% CI 1.6–2.3; Fisher exact test for difference, $p < 0.001$). Interpretation of this value is complicated by the fact that a number of different sampling protocols were used for imported shipments of FDA-regulated foods other than spices and these differences could lead to test sensitivity differences. Comparing only data for shipments that were sampled with the same Category II food sampling protocol as that used for spices (Section 2.1), we find an even larger relative risk for contamination of imported spice shipments as compared with shipments of other imported FDA-regulated foods: 4.4 (95% CI 3.4–5.8; Fisher exact test for difference, $p < 0.001$). The group of other imported FDA-regulated food shipments examined using the Category II food sampling protocol included foods from all but one of the 38 food categories captured in the full set of "all other imported FDA-regulated food" shipments but only 29% of the shipments sampled; the one missing food category (exotic meats) accounted for less than 0.1% of the shipments sampled during this time period. The larger prevalence of *Salmonella* in imported shipments of spices as compared with other FDA-regulated foods is surprising because the low water activity of spices does not support *Salmonella* growth, whereas the high water activity of many other FDA-regulated foods will support growth when other conditions for growth are met (e.g., nutrients and pH; USFDA, 2012g). Further, many spices have inhibitory compounds that provide antibacterial activity against *Salmonella* (Arora and Kaur, 1999; Hammer et al., 1999; Ceylan and Fung, 2004; Indu et al., 2006; Du et al., 2009a, 2009b; Tajkarimi et al., 2010; Hussien et al., 2011). These compounds can limit growth and survival of *Salmonella* in (wet/inoculated) spices and foods containing spices or their essential oils under some conditions (Arora and Kaur, 1999; Hammer et al., 1999; Ceylan and Fung, 2004; Indu et al., 2006; Du et al., 2009a and 2009b; Tajkarimi et al., 2010; Hussien et al., 2011). Obviously other factors, including the ability of *Salmonella* to survive in a variety of low moisture foods including some, if not all, spices (Podolak et al., 2010; Lehmacher et al., 1995), are more important in determining the prevalence of *Salmonella* in imported spice shipments offered for entry to the U.S.

3.1.1. Impact of spice properties

Spices are derived from a variety of plant parts which may result in differences in exposure to pathogen-containing wildlife, insects, and soil during growth, harvest or primary processing. In order to determine whether these differences influence the proportion of imported spice shipments contaminated with *Salmonella*, we grouped spice screening test results by plant part, Table 1. Spices derived from plant seeds, such as cumin, mustard and sesame, or fruit spices, such as black, white, and red pepper, were grouped together in the "fruit" category. Spices derived from plant roots included dried roots, such as turmeric and ginger, as well as dehydrated onion and garlic. Examples of spices included in the leaf category are oregano, basil, and varieties of mint. Examples of spices included in the bark/flower category include cinnamon/cassia, cloves, and saffron. Data for shipments in which the plant part was ambiguous were not included, e.g., shipments described as "coriander" but lacking information as to whether it was the seed or leaf. Prevalence values among the plant part categories ranged from a mean of 0.02 (95% CI 0.0–0.1) for spices derived from the bark/flower of the plant to 0.11 (95% CI 0.068–0.017) for spices derived from plant leaves and differences among some of the categories are significant (chi-square test statistic for multiple proportions (8.8) $>$ chi-square critical value (7.8) at the 95% confidence level). Application of the Marascuillo procedure establishes that a (statistically) larger proportion of imported shipments of both leaf and fruit spices offered for entry to the U.S. are contaminated with *Salmonella* than imported shipments of bark/flower spices. Since 95% of the bark/flower samples examined were either cinnamon/cassia or clove, the difference could arise from reduced test sensitivity for these spices (Section 2.2), the antibacterial activity of these spices against *Salmonella* (Arora and Kaur, 1999; Ceylan and Fung, 2004; Du et al., 2009a; Tajkarimi et al., 2010; Hussien et al., 2011), or differences in growing/processing conditions, including *Salmonella* exposure. Reduced test sensitivity and antibacterial activity against *Salmonella* (Hammer et al., 1999; Burt, 2004; Du et al., 2009b; Tajkarimi et al., 2010) were not sufficient to significantly limit the prevalence of *Salmonella* in imported shipments of oregano and allspice in this study; the shipment prevalence of *Salmonella* for these two spices was 0.12 (95% CI 0.058–0.22).

Salmonella frequency and prevalence in shipments of specific types of imported spices were also evaluated, Table 1. Values are presented for spices for which there were at least 65 shipments examined during the three-year period. In this section of the table, different spices with the same common name, such as coriander seed and leaf, were grouped together. "Capsicum" includes paprika as well as hot and other sweet dried capsicum peppers. In a few cases, we grouped results for different spices together in order to be able to include these data in the table while meeting the required minimum number of shipments. We included the "spices/spices and seasonings, NEC (not elsewhere classified)" category because "NEC" products codes are commonly assigned to imported spice shipments and this category includes less common spices and spice mixtures. Observed prevalence values range from 0.01, for shipments of white pepper (95% CI 0.00–0.06) or the sum of shipments of cinnamon/cassia, clove and nutmeg (95% CI 0.00–0.07), to 0.14 (95% CI 0.083–0.22) for coriander. Application of the chi square test for multiple proportions indicates that the prevalence values for the different types of spices are not all the same (test statistic (50.8) $>$ chi-square critical value (21.03) at the 95% confidence level). However, there is not enough data for each type/category of spice to identify which differences are significant; the Marascuillo procedure did not identify any pairs of spice types that were statistically different. Additional research is needed to distinguish prevalence values among the spice types but these data demonstrate that *Salmonella* shipment contamination is common among a wide range of spice types.

The spice-specific prevalence values in Table 1 can be compared with values determined for these spices in other countries. Moira et al. (2009) found major brands of retail black pepper collected in Botucatu, San Paolo, Brazil between January 2004 and April 2006 to have a statistically larger prevalence (0.18, 95% CI 0.01–0.30, $p < 0.001$) than that found in imported black pepper shipments in this report, even though the Brazilian screening test protocol was less sensitive (examined 25 g as compared with 750 g). While Brazil is a major producer of black pepper, only 3 (1%) of the black pepper shipments examined in the present study were imported from Brazil. Sagoo et al. (2009) also examined large numbers of samples of different spices for *Salmonella* and found a similar range of prevalence values (0.06–0.14) to that shown in Table 1 among many spice types sampled at production and retail sites in the U.K. However, smaller prevalence values for capsicum ($p = 0.031$) and cumin ($p = 0.026$) samples were reported by Sagoo et al. Another investigation in the U.K. found a smaller *Salmonella* prevalence for sesame seeds at retail than that found in this study ($p < 0.001$, Willis et al., 2009). In both U.K. studies, the mass of spice examined in the screening test was smaller than that used in the present study (Sagoo et al., 2009; Willis et al., 2009, Table 2), which could have led to the smaller observed prevalence values. *Salmonella* prevalence values for coriander, black pepper, oregano/basil, and turmeric collected from production and retail sites in the U.K. were not statistically different from the values found in the present study (Sagoo et al., 2009).

3.1.2. Impact of processing

The frequency and prevalence of *Salmonella* in shipments of spices that had undergone different processes, including pathogen reduction treatments, blending, or grinding, are compared to that for spices that had not undergone the process in Table 1. Spice shipments which were classified as "commercially sterile", "heat treated", or "irradiated" or for which the industry supplied product description specified that a pathogen reduction process treatment had been applied to the spice (for example, "steam treated" or "treated with ethylene oxide") were grouped together in Table 1 as "Spices subjected to a Pathogen Reduction Treatment." All other shipments were grouped in "Spices Not Treated/Not known if treated". The small number of spice shipments in this category is not a true reflection of the proportion of imported spice shipments that have been subjected to such treatments because importers are not required to provide this information unless the spice shipment has been irradiated and even in this case, the product code available (USFDA, 2012e) allows importers to choose other ways of defining their product. Therefore, it is likely that the "Spices Not Treated/Not known if treated" group includes spice shipments that had undergone a pathogen reduction treatment prior to U.S. entry.

The observed *Salmonella* prevalence for spice shipments subjected to a pathogen reduction treatment prior to U.S. entry was approximately one-half that for shipments of spices that were not treated or for which no treatment information was provided but the difference is not statistically significant (Fisher Exact Test). The confounding of treated and untreated spice shipments in the "Not treated/Not known" category could be responsible for the similarity in these prevalence values. What is more important is the fact that shipments of "treated" spices were found to contain *Salmonella*. Effective process treatments should not leave any viable *Salmonella* bacteria in the spice. Sagoo et al. (2009) also reported finding "treated" spice samples at retail in the U.K. with unsatisfactory microbiological quality but did not note whether *Salmonella* was found. *Salmonella* contamination of "treated" shipments could reflect ineffective pathogen reduction treatments, very large initial concentrations of *Salmonella* or post-treatment contamination. More research is needed to determine the reason why some process treated imported spice shipments test positive for *Salmonella*.

The *Salmonella* prevalence for shipments of blended spices (mixtures) was statistically similar to that for non-blended spice shipments. Similarly, shipments of ground/cracked spice were not found to have statistically different prevalence values than shipments containing whole spice. While no differences were apparent when comparing the average prevalence for these different categories of spice shipments across all type of spices, significant differences did exist for particular types of spices. For example, prevalence differences were found for shipments of imported ground/cracked capsicum and coriander shipments as compared with their whole counterparts, Table 3, with relative risks of contamination of 11 (95% CI, 2–220) and >10 (95% CI, 2–∞) respectively. In contrast, differences in shipment prevalence were not observed for ground/cracked cumin or black pepper as compared with their whole counterparts, Table 3. In the U.K., Sagoo et al. (2009) found that a larger proportion of spice flakes had unsatisfactory microbiological quality than those in their whole form, but did not specify whether this difference was primarily related to *Salmonella* presence/absence. A larger observed contamination rate for shipments of ground/cracked versus whole spice could arise if the grinding/cracking process introduced contamination and/or if the grinding process dispersed originally highly localized contamination throughout the spice shipment (which would improve the detection efficiency of the screening test). We also note that shipments of whole coriander can include either dried leaf or dried seed whereas shipments of ground/cracked coriander are generally derived from coriander seed. Differences in *Salmonella* prevalence for these different forms of coriander spice could also be responsible for the difference, but this is not expected from the contamination prevalence of spice leaves and fruits reported in Table 1 and Section 3.1.1. Based on product descriptions, at least 63% of the whole coriander shipments examined in this study were coriander seed. More research is needed to determine reason(s) why a larger proportion of imported shipments of ground/cracked capsicum and coriander are contaminated with *Salmonella* than their whole counterparts.

3.1.3. Impact of source country

In order to examine whether the "country of origin" impacts the observed prevalence of *Salmonella* contamination of imported spice shipments offered for entry to the U.S., values were determined for spice shipments imported from different countries without regard to spice type. In most cases, the exporting country is the country where the spice was grown, dried and, if applicable, processed but in some cases, the export country of record is not the country where the spice was grown because the spice shipment was transshipped.

Shipments from 79 different countries were examined during the study period; contaminated shipments came from 37 different countries. *Salmonella* shipment frequency and prevalence values by country are provided in Table 4; only countries for which at least 65 imported shipments were examined are included. Country-specific prevalence values range from 0.009 (95% CI 0.00–0.05) for spice shipments imported from Canada to 0.14 (95% CI 0.086–0.21) for shipments imported from Mexico. Application of the chi-square test for multiple proportions determined that the rates are not all statistically similar and the Marascuillo procedure identified statistical differences between several pairs of countries. A (statistically) larger proportion of spice shipments from India are contaminated than shipments from Canada or Pakistan. The *Salmonella* prevalence for spice shipments exported by Mexico and the 71-country group "All other countries" (which excludes China, India, Indonesia, Mexico, Pakistan, Thailand, and Vietnam) are also larger than that for Canada. Taken together, these data indicate that spice shipments from Canada have a smaller relative risk of contamination than those from many spice exporting countries. Further research is needed to determine the reason(s) for the lower contamination rate for spices imported from Canada but these may include the application of a pathogen reduction treatment to shipments exported to the U.S. Both India and Pakistan are major spice producers. The range of spice types examined from both countries was large, with shipments from 30 different spice categories sampled from India and shipments from 15 different spice categories sampled from Pakistan. Evaluation of *Salmonella* prevalence for shipments among the 14 common spice categories, 0.03 (95% CI 0.01–0.06) for spice shipments from Pakistan and 0.090 (95% CI 0.0.072–0.11) for spice shipments from India, demonstrates that the origin of the difference in *Salmonella* prevalence observed is not related to the importation of unique types of spices from one country as compared with the other.

Willis et al. (2009) found the *Salmonella* prevalence for retail samples of seeds sold in the U.K. was smaller for seeds imported from the European Union (EU) countries than for seeds imported from non-EU countries. Making the same comparison, we find that spice shipments from EU countries did not have a statistically smaller rate of contamination than shipments from non-EU countries, but note that the total number of shipments from these countries was small (79).

Table 4
Frequency and prevalence of *Salmonella*-contaminated imported spice shipments offered for entry to the U.S. as a function of export country, FY2007–FY2009

Exporting country	# positive	*N*	*Salmonella* shipment prevalence	Confidence interval (95%)[a]
Canada	1	110	0.009	0.00–0.05
China	9	245	0.04	0.02–0.07
India	92	1057	0.087	0.071–0.11
Indonesia	2	82	0.02	0.00–0.09
Mexico	19	136	0.14	0.086–0.21
Pakistan	6	205	0.03	0.01–0.06
Thailand	6	111	0.05	0.02–0.1
Vietnam	7	149	0.05	0.02–0.09
All other countries	45	749	0.060	0.044–0.080

[a] 95% exact (Clopper–Pearson) confidence limit (SAS, 2012).

Table 3
Comparison of frequency and prevalence of *Salmonella*-contaminated imported shipments of certain whole and ground/cracked spice offered for entry to the U.S., FY2007–FY2009.

Spice	Whole spice[a]			Ground/cracked spice[a]			RR [95% CI][b]
	# positive	*N*	*Salmonella* shipment prevalence	# positive	*N*	*Salmonella* shipment prevalence	
Capsicums	1	122	0.008	33	366	0.090	11 [2–220]
Coriander	0	43	0.0	16	68	0.24	>10 [2–∞]
Cumin	5	59	0.08	6	79	0.08	0.9 [0.2–3]
Pepper, black	7	156	0.04	6	135	0.04	1.0 [0.3–3]

[a] Categorizations derived from product code (USFDA, 2012e) and description. When description was insufficient to categorize, the sample was not included.
[b] Relative risk of shipment contamination for ground/cracked spice as compared with whole spice; 95% exact (Clopper–Pearson) confidence interval in brackets (SAS, 2012).

3.2. Salmonella serotype diversity in spices

Salmonella serotypes were identified for isolates from most of the contaminated spice shipments (181/187). Multiple serotypes were identified in 12% (22) of the contaminated shipments yielding a total of 205 unique isolates. Ninety-four unique serotypes were identified among these isolates; ninety serotypes were *Salmonella enterica* subspecies enterica, two were *Salmonella enterica* subspecies arizonae, one was *Salmonella enterica* subspecies diarizonae and one was *Salmonella enterica* subspecies salamae, Table 5. *Salmonella* Rissen was not identified in surveillance sampling of spice shipments in the present study despite its association with a large scale outbreak attributed to contaminated

Table 5
Salmonella serotype frequency and percentage among isolates[a] in surveillance samples of imported spice offered for entry to the U.S., FY2007–FY2009.

Serotype	# unique isolates[b]	% of unique isolates[b]	Spice
Weltevreden	13	6.3	Anise, bay, capsicum, coriander, curry powder, onion, sesame seed, spices and seasonings NEC, white pepper
Newport	12	5.9	Capsicum, cumin, curry powder, oregano, sesame seed, spices NEC
Mbandaka	11	5.4	Capsicum, cumin, curry powder, garlic, sesame seed, spices and seasonings NEC
Agona	10	4.9	Anise, black pepper, capsicum, cumin, curry powder, oregano
Bareily	8	4	Capsicum, coriander, cumin, curry powder, fennel, ginger
Montevideo	6	3	Allspice, capsicum, coriander, mint, spices NEC
Typhimurium	6	3	Basil, black pepper, coriander, curry powder, five spice mix,
Anatum	5	2	Capsicum, cumin, sesame, spices NEC
Senftenberg	5	2	Curry powder, sesame seed
Aberdeen	4	2	Ginger, coriander, curry powder
Cubana	4	2	Celery, spices and seasonings NEC
Give	4	2	Capsicum, oregano, sesame seed
Hvittingfoss	4	2	Basil, coriander, spices NEC, turmeric
Mgulani	4	2	Capsicum, spices and seasonings NEC
Rubislaw	4	2	Black pepper, spices NEC
Tennessee	4	2	Capsicum, sesame seed, spices and seasonings NEC
Virchow	4	2	Basil, spices and seasonings NEC, turmeric
Derby	3	1	Black pepper, five spice mix, sage
Enteritidis	3	1	Black pepper, spices and seasonings NEC
Java	3	1	Coriander, mint, spices and seasonings NEC
Poona	3	1	Celery, coriander, turmeric
Sandiego	3	1	Cardamom, coriander, cumin
3,10:b:-	2	1	Capsicum, sesame seed
Bere	2	1	Coriander, spices and seasonings NEC
Bergen	2	1	Curry powder, spices and seasonings NEC
Havana	2	1	Sesame seed, spices and seasonings NEC
Javiana	2	1	Allspice, black pepper
Kentucky	2	1	Cumin, sesame seed
London	2	1	Coriander, fenugreek
Saintpaul	2	1	cumin, mustard
Schwartzengund	2	1	Capsicum, turmeric
S. enterica subspecies diarizonae	2	1	Mint, spices NEC
Barranquilla	1	0.5	Capsicum
Brindisi	1	0.5	Sage
Paratyphi B var. Java	1	0.5	Capsicum
39:z10:z6	1	0.5	Cumin
4,5,12:Rz27:-	1	0.5	Oregano
43:z4, z23:-	1	0.5	Spices NEC
47:z4, z23:-	1	0.5	Curry powder
48:d:z6	1	0.5	Cinnamon/cassia
6, 14:a:1, 5	1	0.5	Spices NEC
6,7,14:e, n, z15	1	0.5	Capsicum
Abaetetuba	1	0.5	Basil
Adabraka	1	0.5	Coriander
Altona	1	0.5	Capsicum
Ball	1	0.5	Black pepper
Bangkok	1	0.5	Spices and seasonings NEC
Bonn	1	0.5	Sesame seed
Braenderup	1	0.5	Black pepper
Brazzaville	1	0.5	Capsicum
Bredeney	1	0.5	Capsicum
Canada	1	0.5	Black pepper
Carmel	1	0.5	Coriander
Carrau	1	0.5	Oregano
Cerro	1	0.5	Sesame seed
Degania	1	0.5	Oregano
Dublin	1	0.5	Curry powder
Eastbourne	1	0.5	Turmeric
Elokate	1	0.5	Black pepper
Freetown	1	0.5	Spices NEC
Gamaba	1	0.5	Cumin
Gaminara	1	0.5	Coriander
Glostrup	1	0.5	Sesame seed
Hermannswerder	1	0.5	Sage
Idikan	1	0.5	Sesame seed
Lexington	1	0.5	Ginger
Llandoff	1	0.5	Sesame seed
Martonos	1	0.5	Capsicum
Minnesota	1	0.5	Basil
Molade	1	0.5	Capsicum
Muenchen	1	0.5	Capsicum

Table 5 (*continued*)

Serotype	# unique isolates[b]	% of unique isolates[b]	Spice
Muenster	1	0.5	Spices and seasonings NEC
Nordrhein	1	0.5	Capsicum
Nottingham	1	0.5	Oregano
Oranienburg	1	0.5	Oregano
Orion	1	0.5	Curry powder
Othmarschen	1	0.5	Spices NEC
Paratyphi B	1	0.5	Turmeric
Potsdam	1	0.5	Sesame seed
Richmond	1	0.5	Spices and seasonings NEC
Siegburg	1	0.5	Turmeric
Simi	1	0.5	Sage
Simsbury	1	0.5	Spices and seasonings NEC
Stanley	1	0.5	Capsicum
Sundsvall	1	0.5	Capsicum
Telelkebir	1	0.5	Cumin
Telhashomer	1	0.5	Fenugreek
Umbilo	1	0.5	Five spice mix
Vejle	1	0.5	Black pepper
Westminister	1	0.5	Sesame seed
Witchita	1	0.5	Spices and seasonings NEC
S. enterica subsubspecies arizonae serotype 48:z4,z24:-	1	0.5	Sesame seed
S. enterica subsubspecies arizonae	1	0.5	Capsicum
S. enterica subsubspecies salamae serotype Degani	1	0.5	Anise

[a] *Salmonella enterica* subspecies enterica unless otherwise noted.
[b] For each spice shipment sampled, the number of unique isolates identified is the number of different serotypes identified. Therefore the number (percent) of isolates is the number (percent) of contaminated spice shipments found with that serotype.

imported white pepper that took place during the study period (CDPH/FDB/ERU, 2010). This observation suggests that *Salmonella* Rissen may not have been a particularly prevalent serotype in spice shipments offered for import into the U.S. during this time period.

The present study establishes that shipments of imported spices can be contaminated by a wide diversity of *Salmonella* serotypes. No single serotype constituted more than 7% of the isolates. Other studies have also reported a wide diversity of serotypes found in spices (Lehmacher et al., 1995; Sagoo et al., 2009; Willis et al., 2009). The observation that a single sample of spice can be contaminated with multiple *Salmonella* serotypes is also not unusual. In one paprika sample, Lehmacher et al. (1995) isolated eleven different serotypes.

Similar serotype diversity has been observed among *Salmonella* isolates from all FDA-regulated imported foods (Zhao et al., 2003, Zhao et al., 2006). Further, the most common serotypes found in spices do not appear to differ substantially from those reported for all FDA-regulated imported foods sampled at import (Zhao et al., 2003, 2006). For example, Weltevreden and Newport were the two most common serotypes isolated from spices during FY2007–FY2009 (present study) and were among the top four serotypes isolated in 2000 and 2001 from examined imported shipments of FDA-regulated foods offered for U.S. entry (Zhao et al., 2003, 2006). These data support the hypothesis that the serotypes most frequently isolated from imported spices are not specific to or preferentially found in spices. A more detailed comparison of serotype prevalence values for spices and other imported FDA-regulated foods is not possible because of the significant differences in sample design between the present study and the studies of Zhao et al. (2003, 2006) and Zhao (2008), where data for spices and compliance samples, such as samples collected as part of an outbreak investigation, were included in the summary statistics. Inclusion of compliance samples in the analysis of serotype prevalence will generally bias values to serotypes associated with the triggering compliance event because multiple samples of the same food source are sampled.

We can also compare the *Salmonella* serotypes isolated from spices offered for import to the United States with those isolated from food samples in other countries. Among the 42 serotypes isolated from food samples collected during 2007–2009 in Asia (a major source of spices for the U.S.) and reported to the World Health Organization Global Foodborne Infections Network (WHO/GFN, 2012), half were also isolated from spices in this study. In contrast with the present spice data, one of the 42 serotypes identified in Asian country food samples accounted for more than half of the isolates, *Salmonella* Infantis from samples collected in Japan. While information on the food associated with each serotype is not available in the WHO/GFN database summaries provided, reports in the literature indicate that *Salmonella* Infantis is commonly isolated from chicken in Japan, e.g., 93.1% of isolates derived from chicken meat samples in Okinawa (Kudaka et al., 2006) and 61.7% of isolates from broiler chickens sampled across Japan (Sasaki et al., 2012).

The serotype diversity observed for isolates from spices offered for import to the U.S. and imported FDA-regulated foods in general, differs in character with that generally observed for isolates from animal meats. Studies of serotype prevalence in raw chicken, turkey, pork, and beef in the United States both at retail and in production, have demonstrated that a small number of serotypes account for the vast majority of *Salmonella* strains isolated from meats and that some serotypes appear to be commodity-specific or preferentially found in a particular commodity (USDA/FSIS, 2012; USFDA, 2012a; Guo et al., 2011; Cui et al., 2005). For example, the 2010 NARMS Retail Meat Report indicated that three *Salmonella* serotypes accounted for 44.5% of the 400 isolates found in retail chicken breast, ground turkey, ground beef and pork chops: Typhimurium, Saintpaul, and Heidelberg (USFDA, 2012f). Most of the Typhimurium isolates (87.8%) were found in chicken breasts while most of the Saintpaul isolates were found in ground turkey (96.0%, USFDA, 2012f). In a similar study in China, Yang et al. (2010) found only 24 different serotypes among the 359 *Salmonella* isolates recovered from retail chicken, pork, beef and lamb samples in Shaanxi China. Six of the 24 serotypes accounted for 81% of these isolates. These data provide some evidence of commodity-specific serotypes in retail meats in China. Among the more common serotypes, Enteritidis comprised 36% of the isolates found in

Table 6
Antimicrobial resistance[a] of *Salmonella enterica* subspecies enterica isolates from FDA surveillance sampling of spices at U.S. Import, FY2007–FY2009.

Serotype	Amikacin	Amoxicillin/Clavulanic acid	Ampicillin	Cefoxitin	Ceftriaxone	Chloramphenicol	Ciprofloxacin
43:z4, z23:-	s	s	s	s	s	s	s
Agona	s	s	R	s	R	R	s
Bareily	s	s	s	s	s	s	s
Bredeney	s	s	s	s	s	s	s
Derby	s	s	s	s	s	R	s
Give	s	s	s	s	I	s	s
Havana	s	s	s	s	s	R	s
Muenster	s	s	s	s	s	R	s
Newport	s	s	R	s	s	s	s
Siegburg	s	s	s	s	s	s	s
Typhimurium	s	I	R	s	s	R	s
Typhimurium	s	s	s	s	s	s	s
Virchow	s	s	s	s	s	s	s
Virchow	s	s	s	s	s	R	s

[a] Resistant (R), Intermediate (I), Susceptible (s), Not Tested (−).

chicken but only 18% of the isolates found in pork, while Derby comprised only 5% of the isolates found in chicken and 37% of the isolates found in pork (Yang et al., 2010). The much wider diversity of *Salmonella* serotypes found in spices may be a reflection of a much wider diversity of contamination sources, such as soil, water, rodents, birds, and insects, as compared with that for animal-derived meat products.

3.3. Antimicrobial resistance of Salmonella in spices

Fourteen (6.8%) of the *Salmonella* isolates from imported spice shipments during the three-year study period were found to exhibit antimicrobial resistance, Table 6. Approximately half (8/14) of the isolates with antimicrobial resistance were found to be resistant to three or more antimicrobials. Two isolates (*Salmonella* serotypes Agona and Newport) were resistant to seven antimicrobials. Perhaps most importantly, approximately one-quarter of the resistant strains (4/14) were resistant to first-line antimicrobial agents used to treat salmonellosis in some populations (Guerrant et al., 2001; Thielman and Guerrant, 2004): trimethoprim/sulfamethoxazole (2) and ceftriaxone (2). None of the isolates were resistant to ciprofloxacin, another first-line antimicrobial for salmonellosis (Guerrant et al., 2001), although many were resistant to nalidixic acid (8/14), which has been found to be an indicator of low level resistance to fluoroquinolones (I. Rodriguez-Avial et al., 2005; Threlfall et al., 2006) and may be a first step towards the development of resistance to ciprofloxacin (Van Looveren et al., 2001). Other common antimicrobial resistances exhibited among the resistant isolates were to sulfisoxazole (10/14), tetracycline (9/14), chloramphenicol (6/14), streptomycin (5/14), kanamycin (4/14) and ampicillin (3/14). No resistance was observed among the isolates to amikacin, amoxicillin/clavulanic acid, or cefoxitin. The isolation of highly resistant *Salmonella* strains from spices has been reported by others (Zhao et al., 2006; Zhao, 2008; Brockmann et al., 2004) including *Salmonella* Typhimurium DT 104, which was involved in the 2001 salmonellosis outbreak associated with sesame seed-helva consumption (Fisher et al., 2001; Brockmann, 2001; Little, 2001; Guérin et al., 2001) and is characteristically resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline (ACSSuT). We observed this phenotype in one isolate each of serotype Typhimurium and Agona.

The prevalence of antimicrobial resistant *Salmonella* strains in spices does not appear to be larger than that found for imported FDA-regulated foods in general, where values of 8–17% were reported for isolates from food samples collected during the years 2001–2005 (Zhao et al., 2003, 2006; Zhao, 2008). The antimicrobial resistance profile of *Salmonella* strains isolated from spice shipments is characteristically different from the resistance profile of strains isolated from retail meats (non-FDA-regulated foods). Antimicrobial resistance is common in retail meats in the U.S. (USFDA, 2012f), Japan (Sasaki et al., 2012 (chickens)) and China (Yang et al., 2010), a major supplier of spices to the U.S. For example, in 2010, 66.5% of the 400 *Salmonella* isolates recovered from retail meats in the U.S. were resistant to one or more antimicrobials (USFDA, 2012f) while 79% of the 359 isolates recovered from retail meats in China during 2007–8 (Yang et al., 2010). Multi-drug resistance is also very common; in 2010 in the U.S., 33.7% of retail ground turkey isolates and 43.3% of retail chicken breast isolates were resistant to three or more antimicrobials (USFDA, 2012f). In China, Yang et al. (2010) found 70% of the isolates were resistant to three or more antimicrobials and 15% were resistant to 13 or more of these drugs. The present study also suggests that *Salmonella* isolates from spices sampled from shipments offered for U.S. entry may be more likely to be resistant to nalidixic acid than isolates from retail meats sampled at retail in the U.S. (1/400; $p < 0.001$; USFDA, 2012f) or China (35/359, $p < 0.001$; Yang et al., 2010). As with the serotype diversity, the smaller antimicrobial resistance profile for spices as compared with retail meats is consistent with a much wider diversity of contamination sources.

4. Conclusions

Spice shipments offered for entry to the U.S. had an overall shipment prevalence for *Salmonella* of 0.066 (95% CI 0.057–0.076) during the fiscal years 2007–2009. This value is approximately twice the value determined for all other FDA-regulated food shipments offered for import into the U.S. (including shipments of fresh produce and ready-to-eat foods) sampled during the same time period. Shipment contamination was found in a wide range of spice types, forms and countries of export; differences in *Salmonella* prevalence were observed among some of these groups. For example, shipments of spices derived from the fruit/seed or leaves of plants had a larger prevalence for *Salmonella* than shipments of spices derived from the bark/flower of spice plants. A larger proportion of ground/cracked capsicum and coriander spice shipments were contaminated than their whole spice counterparts. *Salmonella* shipment prevalence for spices imported from India was larger than that for shipments imported from Canada or Pakistan. The shipment prevalence of *Salmonella* for spices imported from Mexico was also larger than that from Canada. Canadian shipment prevalence of *Salmonella* was also statistically smaller than the mean value for a collection of 71 different countries. No difference in *Salmonella* prevalence was found for shipments of blended spices as compared with non-blended spices. Some spice shipments

Gentamicin	Kanamycin	Nalidixic acid	Sulfisoxazole	Tetracycline	Trimethoprim/Sulfamethoxazole	Streptomycin	Export country	Spice
s	s	s	R	s	s	s	Thailand	Spices NEC
s	R	s	R	R	s	R	Mexico	Oregano
s	s	s	R	s	s	s	Trinidad & Tobago	Curry powder
s	R	R	R	R	s	R	Syrian Arab Republic	Capsicum
s	s	R	s	R	s	s	China (Mainland)	Five spice mix
s	s	R	s	s	s	s	India	Capsicum
s	s	s	s	s	s	s	India	Spices and seasonings NEC
s	s	R	R	R	s	R	Pakistan	Curry mix
R	R	s	R	R	R	R	Mexico	Oregano
s	s	R	s	s	s	s	India	Turmeric
s	s	R	R	R	s	R	Egypt	Basil
s	s	s	R	R	s	s	Pakistan	Curry mix
s	s	R	R	R	R	s	India	Turmeric
s	R	R	R	R	s	s	Egypt	Basil

reported to have been subjected to a pathogen reduction process treatment prior to being offered for U.S. entry were found to contain *Salmonella*.

Spice shipments were contaminated with a wide diversity of *Salmonella* serotypes; 93 unique serotypes were isolated from 205 unique isolates. No single serotype constituted more than 7% of the isolates and some spice shipments were found to contain multiple *Salmonella* serotypes. A small percentage of spice shipments were contaminated with antimicrobial-resistant *Salmonella* strains (8.3%). The wide diversity of serotypes, lack of commodity-specific serotypes, and the small prevalence of antimicrobial resistance found in spices mirrors the findings for other imported FDA-regulated foods, which includes a large percentage of foods from non-animal origin. In contrast, studies in both the U.S. and China have found some *Salmonella* serotypes strongly associated with particular types of retail meats or food production animals. Antimicrobial resistance among *Salmonella* serotypes isolated from retail meats or food production animals also appears to be more common than found in spices, based on studies in the U.S. and China.

Future research should focus on understanding the differences in *Salmonella* prevalence among imported spice shipments to aid in the development of appropriate risk management strategies. Data should also be collected to determine the prevalence of *Salmonella* in spices at retail; these data will provide direct information on the safety of spices consumed and the efficacy of post-import risk management practices currently employed by the spice and food industries and regulatory agencies. Finally, methods development should focus on ways to negate or ameliorate the effects of antimicrobial compounds found in spices, without having to resort to dilution, so that method sensitivity can be improved.

Observations and conclusions made in this study may also apply to dehydrated spice plant material used for other purposes, such as dietary supplements, candy made from seeds, or seeds for sprout production.

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