

Research Paper

Microbiological Quality of Ready-to-Eat Salad Products Collected from Retail and Catering Settings in England during 2020 to 2021

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ABSTRACT

Salad and other fresh produce were collected in England from retail and catering businesses during 2020 to 2021 and were tested for *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), *Listeria*, *Bacillus cereus*, and *E. coli*. Of the 604 samples collected, 57% were from retail settings and 43% were from catering settings; 61% were either salad leaves or salad leaves mixed with other products. Equal numbers of samples were prepacked or loose, and 50% were refrigerated at the time of sampling. Combining results for all microbiological parameters, 84% were interpreted as satisfactory, 12% were interpreted as borderline, and 4% were interpreted as unsatisfactory. One sample (prepacked leaves, cucumber, and tomato from a caterer) was categorized as unacceptable and potentially injurious because of detection of STEC O76; no STEC from human infections in the United Kingdom matched this isolate. No *Salmonella enterica* was detected, but *Listeria monocytogenes* was recovered from 11 samples: 1 at 20 CFU/g and the remainder at <20 CFU/g. *B. cereus* was detected at borderline levels (10^3 to $\leq 10^5$ CFU/g) in 9% of samples and at an unsatisfactory level ($>10^5$ CFU/g) in one sample. *E. coli* was detected in 3% of samples at borderline levels (20 to $\leq 10^2$ CFU/g) and in 4% at unsatisfactory levels ($>10^2$ CFU/g). There was a significant association between detection of *L. monocytogenes* and borderline or unsatisfactory levels of *E. coli*. There were no specific risk profiles associated with products with the higher levels of *B. cereus*, STEC, or *Listeria*, but elevated levels of *E. coli* were predominantly confined to loose products from the United Kingdom collected from caterers in summer or autumn 2021 and may have resulted from relaxation of COVID-19 restrictions. Among the *L. monocytogenes* isolates, only one matched those from human cases and was recovered from a prepacked mixed salad from a catering business in 2021. This isolate was the same strain as that responsible for a multicountry outbreak (2015 to 2018) associated with Hungarian-produced frozen sweet corn; no link to the outbreak food chain was established.

HIGHLIGHTS

- The microbiological quality of 604 samples of salad produce was assessed.
- Of all samples, 84% were satisfactory, 12% were borderline, and 4% were unsatisfactory.
- No *Salmonella* was detected, one sample had STEC, and one had an elevated level of *B. cereus*.
- High levels of *E. coli* were associated with 11 *L. monocytogenes*-contaminated samples.
- One isolate clustered with a previous multicountry outbreak of listeriosis linked to sweet corn.

Key words: *Escherichia coli*; Fresh produce; *Listeria*; Microbiological quality; Salad; Shiga toxin-producing *Escherichia coli*

The consumption of uncooked fruit and vegetables is recognized as a risk factor for foodborne illness (15), and outbreaks of *Listeria monocytogenes*, *Salmonella enterica*, and Shiga toxin-producing *Escherichia coli* (STEC) associated with consumption of salad and fresh products have been described (2, 19, 28, 29). In addition, outbreaks due to other enteric pathogens have been reported, including norovirus, hepatitis A virus, and species within the genera of

Campylobacter, *Shigella*, *Giardia*, *Cyclospora*, and *Cryptosporidium* (1, 15).

Contamination of food of nonanimal origin may result from the use of organic fertilizer or contaminated irrigation water, direct transmission from livestock or wildlife, poor food handling practices, and poor equipment hygiene during and after harvest (15). Extreme weather, including flooding of agricultural fields, poses a further contamination risk, as well as cross-contamination throughout the subsequent food chain (1). A scientific opinion issued by the European Food

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Safety Authority found that food of nonanimal origin was associated with 10% of foodborne outbreaks in the European Union (EU) between 2007 and 2011 (8). Using a model to rank the significance of specific food and pathogen combinations, this report identified that *Salmonella* and leafy greens eaten raw were the top-ranking combination (5).

In 2008, public health risks from preprepared salads in England were reviewed (20). This review considered outbreaks in England and Wales, as well as results from coordinated surveys of the microbiological quality of salad and fresh products. Apart from a survey of salads and sauces in kebab takeaway restaurants in 2007 (25), there has been relatively little analysis of the results from examination of the microbiology of this commodity in England reported in the intervening period.

The purpose of this survey was to investigate the microbiological quality of salad products collected from retail and catering businesses during 2020 to 2021, assess changes in quality over an entire year, and identify links to human infections by comparison to genomes of isolates occurring concurrently in the United Kingdom Health Security Agency (UKHSA, formerly Public Health England) national case registers for United Kingdom human listeriosis, salmonellosis, and STEC infections.

SARS-CoV-2 was recognized as a human pathogen in 2019, and COVID-19 cases increased in the United Kingdom from January 2020. The World Health Organization director-general declared the novel coronavirus outbreak a public health emergency of international concern on 30 January 2020 (34). Subsequently, during 2020 to 2021, there were substantial behavioral changes in the United Kingdom, including food retailing and consumption practices, with closure of places to eat outside the home and general quarantine arrangements (11, 13). Routine physical inspections of food premises by local authorities were reduced during the COVID-19 pandemic period, with an associated reduction in sample collection and microbiological testing (11, 13). Because this survey coincided with the COVID-19 pandemic, an additional aim of this survey was to assist in identifying microbiological changes in the hygiene of salad products during the pandemic period.

MATERIALS AND METHODS

Sample collection. This study used a cross-sectional survey design. Samples of salad and other fresh produce were collected over the period from 30 September 2020 to 9 December 2021 by environmental health practitioners in accordance with the Food Standards Agency food law practice guidance (12). Samples were transported in insulated containers with sufficient frozen material to maintain a temperature between 2 and 8°C. The transport containers all contained temperature-monitoring loggers. All samples were examined by one of the three UKHSA Food, Water & Environmental (FW&E) microbiology laboratories in England, located in London, Porton, or York, which are all designated as official laboratories by the Food Standards Agency in accordance with EU Regulation 2017/625 (7). The microbiological testing commenced the day of or the day after sampling.

Data were collected on each sample by local authority staff using a standardized questionnaire, which included the type, name, and address of the business premises; the date, time, and

temperature of the sample at collection; a sample description and the type of product; the use-by date for the consumer; the country of origin; the packaging type; and whether resampling had occurred from the same premises because of a previous poor microbiological result or because of physical observations of hygiene concerns in the businesses' environment. The information from the questionnaire was recorded via the FW&E Laboratory Information Management System (LIMS) and extracted into Excel spreadsheets. Additional data not captured by the questionnaire were annotated by Internet searches to further classify the settings where samples were collected. To reduce bias on the microbiological results from examination of these foods, products known to be associated with incidents of foodborne illness were not sampled for this survey.

Microbiological examination. Samples of salad were examined using internationally recognized standard methods, comprising detection of *Salmonella* spp. (International Organization for Standardization [ISO] 6579:2017 [all ISO standards available at <https://iso.org>]) by enrichment in Muller-Kauffman tetrathionate novobiocin and Rappaport-Vassiliadis broths and subculture onto xylose lysine deoxycholate and brilliant green agars; detection and enumeration of *Listeria* spp., including *L. monocytogenes* (ISO 11290-1:2017 and 11290-2:2017) by enrichment in half Fraser and Fraser broths and subculture onto chromogenic *Listeria* and Oxford agars; enumeration of the *Bacillus cereus* group (based on British Standard [BS] European Norm [EN] ISO 7932:2004) using a surface spread on mannitol egg yolk agar; enumeration of β -glucuronidase-producing *E. coli* (based on BS ISO 16649-2:2001, using either a surface spread or a pour plate technique) using tryptone bile glucuronic agar; and detection of STEC (ISO Technical Specification [TS] 13136:2012) by enrichment in buffered peptone water, screening by real-time PCR for *stx* and *eae* genes and subculture onto cefixime tellurite sorbitol MacConkey and tryptone bile glucuronic agars. Single samples were examined for each product tested, and all presence or absence tests were performed on 25-g aliquots.

Confirmation of the identity of bacterial isolates was performed in each of the testing laboratories as outlined in the standard methods in the previous paragraph on "Microbiological examination." Microbiological results were interpreted as unsatisfactory, borderline, or satisfactory according to the guidelines for assessing the microbiological safety of ready-to-eat food placed on the market (14). European Commission Regulation 2073/2005 on microbiological criteria for foodstuffs (6) was also used to interpret *L. monocytogenes* and *Salmonella* results (Table 1).

Characterization of *L. monocytogenes* and STEC by whole genome sequencing. Cultures of *L. monocytogenes* and STEC were sent to the Gastrointestinal Bacteria Reference Unit, UKHSA, for confirmation and further characterization, as described previously (3, 4). Pairwise comparisons of single-nucleotide polymorphism (SNP) distances were performed between isolates. SNP profiles were generated based on a seven-threshold sequencing address (3). Isolates of *L. monocytogenes* or STEC strains were defined as being linked if within a 5 SNP single-linkage cluster and were considered to share the same point source such that each isolate had a difference of ≤ 5 SNPs with at least one other isolate within that same cluster. Genomic data were stored in a customized database (Gastro Data Warehouse, London, UK), and pairwise comparisons of SNP addresses was performed on isolates from salad products, cultures from clinical cases that occurred in the United Kingdom, and other isolates from food or

TABLE 1. Interpretive criteria for microbiological results^a

Target	Microbiological quality (CFU/g)			
	Satisfactory	Borderline	Unsatisfactory	Unacceptable or potentially hazardous
<i>L. monocytogenes</i>	<10	10–100	NA	>100
<i>Listeria</i> species ^b	<10	10–100	>100	NA
STEC (culture isolated)	ND ^c	NA	NA	Detected ^{c,d}
<i>Salmonella</i>	ND ^c	NA	NA	Detected ^c
<i>E. coli</i>	<20	20–≤10 ²	>10 ²	NA
<i>B. cereus</i> group	<10 ³	10 ³ –≤10 ⁵	>10 ⁵	NA

^a Guidelines for assessing the microbiological safety of ready-to-eat food placed on the market and European Commission Regulation 2073/2005 (6, 14). NA, not applicable; ND, not detected; STEC, Shiga toxin-producing *Escherichia coli*.

^b Not including *L. monocytogenes*.

^c In 25 g.

^d There is no interpretation of *stx* PCR detected where isolation in pure culture is unsuccessful.

the environment. Sequence data are available through <https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA248549>.

Statistical methods. Descriptive analysis of the data was undertaken using Excel 2010 (Microsoft Corporation, Redmond, WA). Statistical analysis was carried out using the Fisher's exact test, Freeman-Halton probability test (Free Statistics Calculators v. 4.0, <https://www.danielsoper.com/statcalc/default.aspx>) or a chi-square (χ^2) test (Social Science Statistics, <https://www.socscistatistics.com>). A probability value of less than 5% was defined as significant.

RESULTS

Sample origins. Overall, 604 samples were collected by 47 local authorities: 272 (45%) were tested in London, 126 (21%) were tested in Porton, and 206 (34%) were tested in York in FW&E laboratories. Samples were collected during each month of the study, ranging from 8 samples in September 2020 to 97 samples in September 2021. Details of the settings, salad types, countries of origin, and temperature at collection are shown in Table 2. Of the 604 samples, 343 (57%) were collected from retail businesses and the remainder were collected from catering businesses. Most (362 of 604, 60%) were either salad leaves or salad leaves mixed with other products.

Sample storage temperatures. Approximately equal numbers of samples were prepacked or loose, and 50% of all products were, at the time of sampling, stored at <8°C (Table 2). Details of the conditions of storage for the remaining 50% of the samples are provided in Table 2. There was a significant difference between the proportions of samples available as loose and those available prepacked among the different settings (χ^2 test = 290, $P < 0.05$): 89% of samples from catering premises were loose (i.e., not packaged at the time of sampling); in contrast, for samples from supermarkets and other retailers, 89 and 64%, respectively, were prepacked.

There were significant differences in the collection of data on temperature of storage among the different settings (χ^2 test = 30, $P < 0.05$): the storage temperature was not recorded for 92% of samples from catering premises but

was recorded for 77% of samples from supermarkets and 78% of samples from other retail establishments. Where temperature of storage data were available, there were significant differences among the settings (χ^2 test = 15, $P < 0.05$). Among all settings, 57 to 66% of samples were recorded as being stored at <8°C. In contrast, 15, 24, and 15% of samples from supermarkets, other retail settings, and catering businesses, respectively, were collected at temperatures of at ≥15°C or at ambient temperature. Furthermore, 22, 10, and 28% of samples from supermarkets, other retail settings, and catering businesses, respectively, were collected from 8 to <15°C.

Sample durability. Durability data were recorded for 300 (50%) of the 604 samples (Table 3), with no samples identified as beyond their specified use-by date. The availability of durability data varied significantly among the settings (χ^2 test = 105, $P < 0.05$) and was available for 39% of the 261 samples from catering businesses, 75% of the 171 samples from supermarkets, and 22% of the 26 samples from other retailers. Durability dates were significantly more common in prepacked compared with loose products (χ^2 test = 48, $P < 0.05$): 35% of products sold loose had durability dates compared with 65% of the prepacked products. There were insufficient data to establish whether durability dates were equally available for the different types of packaging among the settings. There was no significant difference between the proportions of samples with ≤2 days compared with those with >2 days of shelf life remaining among the different settings (χ^2 test = 1.9, $P = 0.4$). However, there was a significant difference among the settings in the percentages of samples with ≤5 days compared with >5 days of shelf life remaining (Freeman-Halton probability test, $P < 0.05$), with most samples from catering settings (99%) and supermarkets (93%) within ≤5 days of shelf life remaining compared with 77% of samples from other retail establishments (Table 3).

Country of origin. Country of origin data were available for 47% of the products: of those, 89% were

TABLE 2. Types of products, packaging, and temperature at collection of 604 ready-to-eat salad products collected from retail and catering settings in England during 2020 to 2021

Category of sample	No. (%) of samples
Setting	
National chain supermarkets	227 (38)
Other retail	116 ^a (19)
Catering	261 ^b (43)
Type of product	
Salad leaves	201 (34)
Salad leaves mixed with other products	161 (27)
Tomato	60 (10)
Fresh herbs	40 (7)
Cucumber	35 (6)
Carrot	18 (3)
Pepper	18 (3)
Onion	15 (2)
Cabbage	11 (2)
Other	45 ^c (7)
Type of packaging	
Prepacked	284 (47)
Loose	285 (47)
Not recorded	35 (6)
Temp at collection	
<8°C	303 (50)
8–<15°C	115 (19)
15–25°C	40 (7)
Ambient	44 (7)
Chilled	3 (<1)
Not recorded	99 (16)
Collected as part of resampling^d	
No	525 (87)
Yes	35 (6)
Not recorded	44 (7)

^a Butcher (10), baker (1), small shop (68), farm shop (35), market (2), petrol station (1).

^b Café (66), hospital (3), hotel (11), takeaway restaurant (131), public house (9), restaurant (41).

^c Avocado (3), beans (1), beetroot (1), celery (2), chicory (1), chilli (2), coleslaw (2), fennel (1), flowers (4), mushroom (1), radish (7), sprouted seeds (8), sweet corn (8).

^d Resampling from the same premises because of a previous poor microbiological result or because of physical observations of hygiene concerns in the businesses' environment.

TABLE 3. Time between sampling and use-by date for salad products collected from retail and catering settings in England during 2020 to 2021

	No. (%) of samples			
	Use-by date recorded	≤2 days	≤5 days	>5 days
National chain supermarkets	229	58 (25)	158 (69)	13 (6)
Other retailers	40	14 (35)	20 (50)	6 (15)
Catering settings	189	86 (45)	102 (54)	1 (1)
All samples	458	158 (34)	280 (61)	20 (4)

from either the United Kingdom or the EU (Table 4). The country of origin was markedly seasonal, with products from the United Kingdom predominating in the final quarter (October to December) of 2020 and the third quarter (July to September) of 2021. Products imported from both the EU and elsewhere were markedly more common during January to June 2020 and October to November 2021 (Table 4).

Resampling. Among all samples, 35 (13%) were collected (resampled) from the same premises following previously adverse results or physical observations of hygiene concerns in the businesses' environment (Table 2). The time between the primary sampling and the resampling varied from 2 weeks to 7 months. There was a significant difference in the proportion of resampling undertaken among the settings (Fisher's two-tailed exact test, $P \leq 0.05$). Of the 35 resamples, 26 were from 15 catering establishments, 2 were from two national chain supermarkets, and 7 were from one other retail premises, which was a small shop. These resampled establishments constituted 10% of the total catering businesses compared with 1% of the national chain supermarkets and 6% of the other retail premises. The 15 catering settings where resampling was performed were five takeaway restaurants, four other restaurants, four cafés, and two public houses.

Results of microbiological testing. Results of microbiological testing for each of the target bacterial groups or parameters are shown in Table 5. All samples were tested for each target except that 16 samples were not tested for *B. cereus*. Combining the results for all six parameters, 509 (84%) were of a satisfactory microbiological quality, 70 (12%) were of a borderline quality, and 23 (4%) were of an unsatisfactory quality (Table 5). Only one sample (contaminated with STEC) was considered unacceptable and potentially injurious. *L. monocytogenes* was detected in 11 samples (1 at 20 CFU/g and the remainder at levels <20 CFU/g). *Listeria* species other than *L. monocytogenes* were detected in 19 samples. One sample was contaminated at borderline levels (100 CFU/g) with *Listeria innocua*, and one of the samples contaminated with *L. monocytogenes* was also contaminated with *Listeria seeligeri*; both were at <20 CFU/g. *B. cereus* was detected at borderline levels in 9% of samples and at an unsatisfactory level (>150,000 CFU/g) in one sample. *E. coli* was detected at borderline levels in 21 samples (3%) and at unsatisfactory levels in 23 samples (4%, with counts ranging from 120 to >3,000 CFU/g).

The two samples categorized as borderline for either *L. monocytogenes* or *Listeria* species also had borderline levels of *E. coli*. There was a significant association between the presence of *L. monocytogenes* and *Listeria* spp. with borderline or unsatisfactory levels of *E. coli* (χ^2 test = 36 and 32, respectively; $P < 0.05$). Of the 11 samples with elevated levels of *E. coli* and any *Listeria* species, 9 were collected from catering environments. Two samples contaminated with *B. cereus* at borderline levels were also contaminated with borderline or unsatisfactory levels of *E. coli*. All samples with *L. monocytogenes* or other *Listeria*

TABLE 4. Seasonal distribution by country of origin for 281 salad products collected from retail and catering settings in England during 2020 to 2021

Country of origin	Total (%)	No. of samples collected (% for each period)				
		Sep.–Dec. 2020	Jan.–Mar. 2021	Apr.–June 2021	July–Sep. 2021	Oct.–Nov. 2021
United Kingdom	157 (56)	30 (94)	29 (34)	15 (38)	72 (91)	11 (24)
EU	92 ^a (33)	0	38 (44)	17 (44)	6 (8)	31 (69)
Imported from outside of the EU	32 ^b (11)	2 (6)	19 (22)	7 (18)	1 (1)	3 (7)
Total where country of origin was known	281 ^c	32	86	39	79	45
<i>E. coli</i> ^d		2 (2)	1 (<1)	6 (6)	30 (16)	5 (12)
<i>B. cereus</i> ^d		14 (12)	20 (13)	7 (7)	9 (5)	2 (5)
Total tested		113	165	94	191	41

^a Belgium (1), France (2), Greece (1), Italy (18), The Netherlands (20), Poland (2), Portugal (3), Republic of Ireland (2), Spain (43).

^b China (1), Ethiopia (1), India (4), Israel (1), Kenya (6), Morocco (15), Senegal (1), South Africa (1), United States (2).

^c For 323 samples, country of origin was not recorded.

^d At levels interpreted as borderline or unsatisfactory.

species contained satisfactory levels of *B. cereus*. The sample from which STEC was recovered and the sample in which *B. cereus* was detected at unsatisfactory levels were both considered satisfactory for all other parameters.

The distribution of borderline and unsatisfactory *B. cereus* and *E. coli* levels, together with the presence of *Listeria* and STEC, among food types, packaging type, settings, and country of origin is shown in Table 6. The STEC isolate was from a prepacked sample of green leaves, cucumber, and tomatoes collected from a catering setting.

Markers of microbiological quality showed different distributions across the different categories. Borderline and unsatisfactory levels of *B. cereus* were detected from a range of salad types, found in similar proportions among loose and prepacked products, and found equally in samples from catering businesses, across different retail settings, and from different countries of origin. In contrast, although borderline and unsatisfactory levels of *E. coli* were similarly distributed across salad products, they were predominantly confined to loose products collected from catering settings with a United Kingdom country of origin. Among the 11 samples in which *L. monocytogenes* was detected, 5 were recovered from loose products taken from two premises (see

the next section). Excluding the resampled products, *L. monocytogenes* showed a similar distribution to that of *Listeria* spp. in that they were predominantly detected in samples of salad leaves, were recovered from twice the number of loose compared with prepacked products, and were recovered from similar proportions of samples from catering and retail settings.

There were insufficient products tested to detect seasonal effects for the presence of *Listeria* or STEC. The detection of *E. coli* at borderline or unsatisfactory levels was significantly more common in samples from June to November (χ^2 test = 22, $P < 0.05$), particularly in 2021 (Table 4). All samples with borderline or unsatisfactory levels of *E. coli* between July and November 2021 were collected from catering establishments. In contrast, the presence of *B. cereus* at levels interpreted as borderline or unsatisfactory was significantly more commonly detected in winter 2020 and spring 2021 (χ^2 test = 5.5, $P < 0.05$). During winter 2020 and spring 2021, 80% of the samples with borderline or unsatisfactory levels of *B. cereus* were collected from retail settings. There were insufficient products tested to further investigate the seasonal effects on the presence of *E. coli* or *B. cereus*.

TABLE 5. Results of *Bacillus cereus*, *E. coli*, *L. monocytogenes*, *Listeria species*, and STEC detected in 604 salad products collected in England during 2020 to 2021

Target	No. (%) of samples			
	Satisfactory	Borderline	Unsatisfactory	Unsatisfactory or potentially hazardous
All targets	509 (84)	70 (12)	24 (4)	1 (0.2)
<i>L. monocytogenes</i>	603 ^a	1 (0.2)	0	0
<i>Listeria</i> species (other than <i>L. monocytogenes</i>)	603 ^b	1 (0.2)	0	0
STEC (culture isolated)	603	0	0	1 (0.2)
<i>Salmonella</i>	604	0	0	0
<i>E. coli</i>	560	21 (3)	23 (4)	0
<i>B. cereus</i>	536 ^c	51 (9)	1 (0.2)	0

^a *L. monocytogenes* was detected at <20 CFU/g in 10 samples.

^b *Listeria* species were detected at <20 CFU/g in 19 samples.

^c Levels of *B. cereus* were not available for 16 samples.

TABLE 6. Distribution of adverse results for the detection of *Bacillus*, *E. coli*, *L. monocytogenes*, *Listeria species*, and *STEC* among salad types, packaging types, settings, and countries of origin of 604 salad products collected in England during 2020 to 2021

Category	No. of samples generating results within each category (% of total within each category)				
	<i>Bacillus</i> , borderline and unsatisfactory	<i>E. coli</i> , borderline and unsatisfactory	<i>L. monocytogenes</i> , all detections	<i>Listeria species</i> (excluding <i>L. monocytogenes</i>), all detections	STEC
Total	52	44	11	20	1
Food types					
Salad leaves	19 (37)	13 (30)	6 (55)	7 (20)	0
Salad leaves mixed with other products	10 (19)	14 (32)	5 (45)	11 (55)	1
Tomato	11 (21)	3 (7)	0	0	0
Other	12 ^a (23)	14 ^b (32)	0	2 ^c (10)	0
Packaging type					
Loose	18 (35)	35 (80)	7 (64)	13 (65)	0
Prepacked	29 (56)	8 (18)	4 (36)	6 (30)	1
NK ^d	5 (10)	1 (2)	0	1 (5)	0
Setting					
Catering	15 (29)	39 (89)	9 (82)	11 (55)	1
National chain supermarkets	22 (42)	3 (7)	1 (9)	5 (25)	0
Other retail	15 (29)	2 (4)	1 (9)	4 (20)	0
Country of origin					
United Kingdom	13 (25)	23 (52)	2 (18)	4 (2)	1
EU	12 (23)	0	0	2 (10)	0
Other	3 (6)	1 (2)	0	1 (5)	0
NK	24 (46)	20 (45)	9 (82)	13 (65)	0
Collected as part of resampling					
No (all samples)	43 (83)	30 (68)	8 (73)	17 (85)	1
No (only primary samples ^e)	6 (12)	18 (41)	2 (18)	4 (20)	0
Yes	1 (2)	7 (16)	3 (27)	0	0
NK	8 (15)	7 (16)	0	3 (15)	0

^a Fresh herbs (6), cucumber (3), sprouted seeds (1), pepper (2).

^b Fresh herbs (1), cucumber (1), carrot (3), pepper (2), cabbage (3), onion (2), sweet corn (2).

^c Cabbage (1), mushroom (1).

^d NK, not known.

^e Results from 30 primary samples collected from the same 18 premises as the 35 resampled products.

A comparison of all samples with those collected as part of resampling showed that there was a reduction in the percentages of samples with borderline or unsatisfactory levels of *E. coli* or *B. cereus* in the products subsequently collected from the same businesses (Table 6). Furthermore, detection of both *L. monocytogenes* and *Listeria* spp. was reduced in resamples compared with the original samples (Table 6). The 35 resampled products were collected from 18 food premises associated with 30 primary samples previously collected from the same premises. Overall improvement in microbiological quality was observed between the primary and the resampled samples, with percentages of satisfactory, borderline, and unsatisfactory results from the businesses of 27, 40, and 33% for the primary samples, compared with 77, 11, and 11% for the resampled samples, respectively. Reductions were associated with *E. coli* and *B. cereus* counts, as well as *Listeria* spp. occurrence between the primary and the resampled products.

Characterization of *L. monocytogenes*, *Listeria* spp., and *STEC*. Of the 11 *L. monocytogenes* isolates, all were recovered during 2021 and eight distinct strains detected, designated A to H (Table 7). Five of these were unique *L. monocytogenes* strains (designated D to H), all detected at <20 CFU/g and recovered from different products collected from unrelated retail or catering establishments. Strain A was recovered from three loose samples of mixed salad (cut lettuce, tomato, and cucumber or just cut lettuce) collected from the same catering establishment (a takeaway salad bar) on two occasions in July and October 2021: the strain was recovered at 20 CFU/g in one sample and at <20 CFU/g in the remaining two samples. *L. monocytogenes* strain A was also recovered from eight environmental swabs (the sink used to wash salad, a colander, and chopping boards) collected from the same premises in September and October 2021.

L. monocytogenes strain B was recovered from two loose salad samples (cut lettuce and sliced onion and

TABLE 7. Characterization of *L. monocytogenes* isolated from 604 salad products collected in England during 2020 to 2021^a

Setting ^b	Month of collection in 2021	SRA accession no.	Serogroup	ST	CC	Strain
Catering 1	July	SRR15376353	1/2b	5	CC5	E
Catering 2	July	SRR15376347	4	1	CC1	A
Catering 2	July	SRR15843279 ^c	4	1	CC1	A
Catering 2	Oct.	SRR16951766	4	1	CC1	A
Catering 3	July	SRR13645789	4	2	CC2	H
Catering 4	Aug.	SRR15897255	4	6	CC6	C ^d
Catering 5	Aug.	SRR15842420	1/2a	37	CC37	B
Catering 5	Aug.	SRR15842509	1/2a	37	CC37	B
Catering 6	Oct.	SRR16641546	4	1	CC1	G
Retail 7	May	SRR14795845	1/2a	91	CC14	D
Retail 8	Aug.	SRR16053781	4	1	CC1	F

^a Strains were defined on the basis of being linked within a 5 single-nucleotide polymorphism (SNP) single-linkage cluster and were considered part of the same point source, and each isolate had a difference of no more than 5 SNPs with at least one other isolate within that same cluster. SRA, short read archive; ST, sequence type; CC, clonal complex.

^b Separate establishments are designated by numerical suffixes.

^c All detected at <20 CFU/g except this culture, which was present at 20 CFU/g.

^d This strain was associated with a multicountry outbreak linked to Hungarian sweet corn consumption (9, 26). No other matches with isolates from human cases of listeriosis in the United Kingdom were detected (2015 to 2022).

tomato) at <20 CFU/g from a refrigerated display in a restaurant on the same day in August 2021.

L. monocytogenes strain C formed a monophyletic cluster of <5 SNPs with isolates from a multicountry outbreak of listeriosis associated with Hungarian-produced sweet corn consumption between 2015 and 2018 (9, 22). This strain was recovered at <20 CFU/g from a bag of prepacked mixed salad (bistro salad containing lettuce and beetroot) collected from a catering establishment that had purchased this from a national supermarket chain in August 2021.

Listeria species other than *L. monocytogenes* were detected in 20 of the salad samples: *L. innocua* from 11 samples, *L. seeligeri* from 5 samples, and *Listeria welshimeri* from 2 samples, whereas the final 2 samples were not *L. monocytogenes* but were identified only to the *Listeria* genus. Three of the *L. innocua* samples were recovered from three salad products (salad leaves, salad leaves plus cucumber, and onion and cabbage) collected on the same day from a single catering establishment: *L. innocua* was detected in the sample of salad leaves at 100 CFU/g, and the other two products were contaminated at <20 CFU/g. In a second catering establishment, a takeaway restaurant, *L. innocua* was detected in two samples of salad leaves from a refrigerated display (both at <20 CFU/g). No further characterization was carried out on any *L. innocua* isolates from related establishments.

The STEC isolate was from a prepacked mixed salad (green leaves, cucumber, and tomatoes) collected from a public house. The isolate was characterized as O76:H19, ST675, *stx*_{1c} positive, and *eae* negative (National Center for Biotechnology Information Sequence Read Archive SRR references 16466308 and 16466339; UKHSA BioProject PRJNA315192, <https://www.ncbi.nlm.nih.gov/sra/SRR16466308/> and <https://www.ncbi.nlm.nih.gov/sra/SRR16466339/>). Further sampling of the same salad

product from the same premises took place approximately 4 weeks later; no STEC was detected.

DISCUSSION

Public health risks, including outbreaks of listeriosis, salmonellosis, and STEC infections, have previously been identified (1) as associated with the types of salad products tested here in England during 2020 to 2021. Of the 604 samples tested, and combining results for all microbiological parameters, 84% were interpreted as satisfactory, 12% were interpreted as borderline, and 4% were interpreted as unsatisfactory. One sample (prepacked green leaves, cucumber, and tomatoes collected from catering businesses) was categorized as unacceptable and potentially injurious because of the isolation of STEC O76:H19 with Shiga toxin gene *stx*_{1c}.

Salads and public health risks in England were previously reviewed in 2008 (20). This 2020 to 2021 study showed 1.7% contamination with *L. monocytogenes* and 4% contamination with *E. coli* at levels of >100 CFU/g. Among more than 9,000 samples of similar food tested between 1995 and 2001, contamination by *L. monocytogenes* varied between 2 and 3%, and *E. coli* was at levels of >100 CFU/g in 0.3 to 1.5% (20). It is gratifying that results from the 2020 to 2021 study indicate improvements in microbiological quality when sampling officers resampled from businesses that previously gave poor results. However, we did not detect overall improvement in microbiological quality compared with similar products sampled in England 20 to 25 years previously (20). The proportion of salad samples with levels of *Listeria* (including *L. monocytogenes*) at levels of >20 CFU/g were previously reported as 0.2% (21, 27, 28), which was higher than that reported here. However, unlike here, most salad samples with elevated levels of *Listeria* were salads with the addition of meat (particularly chicken) or seafood (21). Furthermore, a survey of food from takeaway restaurants in the United

Kingdom in 2007 detected *E. coli* at levels of >100 CFU/g in 6% of cucumber samples and in 5% of lettuce samples (25). *Salmonella* was not recovered from any sample described here, consistent with previous studies, with the exception of one older United Kingdom study in 2001, in which *Salmonella* was recovered from 5 of 3,852 bagged salad samples that originated from two growers (28) and 1 of 454 samples from takeaway restaurants (25).

Among all samples described in this 2020 to 2021 study, 9 and 0.2% of samples were classified as borderline and unsatisfactory, respectively, because of the presence of elevated levels of the *B. cereus* group. There are few published data to compare this with, and we carried out only limited further characterization to clarify the risks associated with these levels of contamination. No specific risk profiles could be identified associated with the higher levels of *B. cereus*, which occurred in a range of salad types and was found in similar proportions among loose and prepacked products, as well as in samples from catering and retail settings and different countries of origin. Samples with higher *Bacillus* levels were more often collected during winter 2020 and spring 2021, but there were insufficient data for further investigation. These results may at least partly reflect the presence of *Bacillus thuringiensis*, which can be used as a pesticide on crops, but the testing done was unable to distinguish *B. thuringiensis* within the *B. cereus* group. *B. thuringiensis* can cause gastroenteric illness if present at high levels, and it was therefore considered appropriate to interpret all *B. cereus* group results according to the criteria indicated in the Health Protection Agency guidelines for ready-to-eat food (14), regardless of determining the exact species.

The risks for elevated levels of *E. coli* contamination differed from those for *B. cereus*, and although similarly distributed across different salad products, they were predominantly confined to loose products with a United Kingdom country of origin and collected from catering settings in summer and autumn 2021. We speculate that these are more likely to be associated with environmental contamination and may have resulted from the relaxation of COVID-19 restrictions.

We previously reported an association between elevated levels of *E. coli* and both *L. monocytogenes* and other *Listeria* species in cooked chicken (23). Similarly, in the study described here, an association was observed between *E. coli* and *Listeria* in salad products. No other associations between any microbiological parameters investigated here were detected. We did not investigate possible causes for the association between *E. coli* and *Listeria* for the samples, but cross-contamination and poor hygiene in catering environments are likely to be major factors. Despite the widespread use of *E. coli* and *Listeria* as indicators of poor hygiene (26), there are often limitations in understanding the causes of the presence of these organisms at elevated levels. Previous investigations have commented on the effectiveness of food handler training on the maintenance of microbiological quality of salad vegetables (27), and we believe that there are further opportunities for investigation of how indicator organisms relate to the effectiveness of food safety management systems, including predicting

contamination of ingredients at the point of production and the effectiveness of cleaning and hygiene in all production environments.

We have used similar cross-sectional survey designs in previous English surveys (18, 23, 24, 32, 33), which have generated risk-based data that have proven value for public health microbiology. These surveys are part of the routine surveillance of food by authorities, and the collection of food is often part of their regulatory inspection regime. Approximately 48,000 samples per year were collected by practitioners in local authorities and tested by UKHSA before the COVID-19 pandemic. Because of the pandemic, there was a marked reduction in activity, with 23,500 samples received in 2020 and 28,000 samples received in 2021. For the study described here, a protocol was produced for practitioners with standardized transport and testing methodologies in the UKHSA FW&E microbiology laboratories. Information was available from the single LIMS. To reduce bias, samples already identified as associated with incidents of human infection were not included. The studies therefore reflect investigation and sampling from premises based on local risk-based considerations: for example, in the study described here, a higher number of samples from catering settings (particularly takeaway restaurants and cafés) were submitted that may be perceived as representing greater microbiological risks compared with the number from larger retailers, e.g., large chain supermarkets.

There are limitations with this study design in that sampling was not strictly coordinated with a rigid study design (for example, based on market share). However, results from cross-sectional studies (including those reported here) show many similarities and have identified trends similar to those generated from more formal study designs, such as those based on market share (16, 17, 31). In addition, cross-sectional studies may be more informative for public health purposes, because a disproportionate number of infections caused by a restricted range of food producers and smaller producers (which sometimes represent a greater public health risk) may not be sampled using a market share approach.

Using whole genome sequencing data, apart from one *L. monocytogenes* isolate, all cultures from the salad products analyzed were distinct from those recovered from human cases in the United Kingdom considered as not having a recent common ancestor. However, one *L. monocytogenes* isolated from salad in 2021 was identified as the same strain as that associated with 12 listeriosis cases that occurred in the United Kingdom between 2015 and 2021 and were part of a multicountry outbreak attributed to consumption of frozen Hungarian-produced sweet corn (9, 22). An international recall of the contaminated sweet corn took place in 2018, and further sweet corn contaminated by the outbreak strain was detected in England at the importer in 2019. There was no information available on the countries of origin for the salad components in the salad product tested in 2021, and despite tracing investigations, no link to the Hungarian sweet corn food chain was established. Two further cases of listerial meningitis were detected in England as infected by this strain after the end of

the outbreak in 2018. The outbreak strain was detected in the cerebral spinal fluid (CSF) of a patient in 2019 who also had contaminated sweet corn in a domestic freezer (22). A second case of human meningitis was detected in England in 2020, where the outbreak strain was recovered from this patient's CSF. The 2020 patient had a history of eating frozen sweet corn, but there was no relevant product available for testing and no attribution of infection to a specific food exposure was established (30). No further cases infected by the outbreak strain were detected in the United Kingdom at the time of writing this report (30). Previous testing in 2018 showed that a range of frozen vegetables from the Hungarian food producer, including sweet corn, was contaminated by the outbreak strain (9). *L. monocytogenes* can persist within food production environments, including individual pieces of equipment, for decades (10). International surveillance personnel should be aware of the detection of this *L. monocytogenes* outbreak strain in a ready-to-eat food in summer 2021 and investigate food exposures in subsequent listeriosis cases infected by this type.

In this study, there was one sample classified as potentially injurious because of contamination by STEC O76:H19, ST675, *stx*_{1c} but *eae* negative. None of the STEC isolates of this serotype from the clinical cases in the UKHSA archive were within 100 SNPs of the isolates from the salad described here. Although no direct public health incidents are known to be associated with the isolate from this salad product, this observation illustrates the potential for this broad food commodity to be a source of transmission. There were 101 human cases in which STEC O76:H19, ST675, was isolated from specimens of feces in the United Kingdom between 2014 and 2021; *eae* was not detected in any of these, and 32 patients reported diarrhea, 2 patients with bloody diarrhea. Data on symptoms for the remaining cases were unavailable.

Sampling for this study proved more difficult than on previous occasions because of the COVID-19 pandemic. Most assessments of food businesses by local authorities were performed remotely (11, 13). Consequently, there were reduced levels of food sampling and testing compared with previous studies over similar periods (20). Sampling practicalities during the pandemic might explain the lack of some data collection, such as temperature of storage, particularly in retail settings. In addition, there were likely to be changes in the food chain as a result of the COVID-19 pandemic in terms of shopping, catering, and eating practices (14), as well as the sourcing of specific food commodities. The countries of origin also appeared to vary during the pandemic period, with a larger proportion of samples being sourced from the United Kingdom during autumn 2020 compared with products being more commonly imported from other countries during the same period of 2021. These differences may reflect changes in supply chains as a result of the pandemic, as well as a consequence of the United Kingdom's EU exit. Furthermore, there were relaxations in restrictions during summer 2021 such that more eating outside the home occurred, together with reopening of catering establishments with consequential staffing changes (13). These changes may

have contributed to the poorer microbiological quality of products sampled in catering settings in summer and autumn 2021, although ambient temperature may also have been a contributing factor. Therefore, some further verification of the microbiological quality of salad products after the pandemic has ended may be needed because of atypical features of the food chain during the study period.

This study analyzed the microbiological quality of 604 salads and other fresh produce collected in England from retail and catering premises from September 2020 to December 2021. Data have been presented on likely trends and seasonal differences in the sourcing of specific salad commodities, as well as practices likely to affect hygiene in different settings. The results from this study do not suggest that there have been improvements in microbiological quality since previous studies over the past 20 to 25 years. However, the results presented here show that 84% of ready-to-eat salad products were of good microbiological quality, with <5% giving unsatisfactory results. These results indicate areas for improvement in hygiene during production and handling of salad items, particularly in the catering sector.

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