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Survey of the microbiological safety of ready-to-eat, pre-cut and pre-packaged fresh herbs and salad leaves from retail establishments in Ireland (13NS7)

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SUMMARY

This survey investigated the microbiological safety of ready-to-eat, pre-cut and pre-packaged fresh herbs and salad leaves available at retail sale in Ireland. Over 1,000 samples were tested for the presence of *Salmonella* and enumerated for *Listeria monocytogenes*. *Salmonella* was detected in only 0.1% (1/1,005) of samples; this was a bag of rocket leaves grown in Italy from which *S.* Napoli was isolated. *L. monocytogenes* was below the limit of enumeration (<10 cfu/g) for 99.8% (998/1,000) of samples and at 10 cfu/g for the remaining two samples, all well below the maximum legal limit of 100 cfu/g.

Some samples were tested for the presence of verotoxigenic *E. coli* (VTEC). In total, 0/247 samples tested using the CEN/ISO TS 13136 method (which targets the major VTEC virulence genes *stx* and *eae*) were positive. In addition, 0/397 samples tested specifically for *E. coli* O26 were positive and although 1/403 samples tested specifically for the presence of *E. coli* O157 was positive, the isolate did not contain the genes required to produce verotoxin and therefore, was not of clinical significance.

This survey was carried out from June to October, the months when Irish produce was most likely on sale. Irish origin produce made up 62% of the samples tested in this survey, none of which were unsatisfactory.

Producers labelled fresh herbs and salad leaves with a wide range of storage instructions, particularly in relation to the temperature for chilled storage. Maximum storage temperatures as low as 3ºC were recommended by some producers; however, the national recommended temperature for chilled storage is 0-5ºC. Food business operators that package ready-to-eat, pre-cut, fresh herbs and salad leaves should also be aware that the temperature in domestic fridges is generally higher than at retail and wholesale level.

In total, 87% of samples were stored or displayed in refrigerated conditions at the time the sample was collected. The air temperature of the refrigeration unit for the majority (77%) of these samples was ≤5ºC. However, the air temperature of the refrigeration unit for 23% of chilled samples was >5ºC. Indeed, an air temperature of 7.1ºC was measured for the refrigeration unit in which the *Salmonella*-positive bag of rocket leaves was stored. This temperature could allow *Salmonella* numbers on the already contaminated product to increase if the shelf-life is sufficiently long. Food business operators should ensure that refrigeration units do not exceed the maximum chilled temperature of 5ºC.

The bag of rocket in which *Salmonella* was detected was labelled as already washed. Washing (with or without the presence of sanitisers) cannot eliminate pathogens on fresh produce. Therefore, producers must take all reasonable measures to control potential points of contamination in the field, during harvesting, processing and distribution; for example using guides to good practice such as the Food Safety Authority of Ireland (FSAI) Code of Practice for Food Safety in the Fresh Produce Supply Chain (FSAI, 2001a). In addition, food business operators should ensure that their traceability records for the fresh herbs and salad leaves are robust, as this will facilitate rapid control measures to be implemented should a pathogen be detected in a batch of fresh herbs or salad leaves or if they are implicated in an outbreak of illness. The FSAI has produced Guidance Note No.10 on Product Recall and Traceability (FSAI, 2013).

ACKNOWLEDGEMENTS

The FSAI thanks those that participated in this survey: environmental health officers (EHOs) and the laboratory staff of the seven official food microbiology laboratories of Health Service Executive (HSE)[1]; the National Listeria monocytogenes Reference Laboratory (Backweston); and the National Salmonella, Shigella and Listeria Reference Laboratory (NSSLRL) in Galway.

INTRODUCTION

The health benefits of eating fresh fruit and vegetables are well recognised, with consumers in Ireland encouraged to eat five or more portions a day (Department of Health, 2012). While most consumers in Ireland are well-informed about the health benefits from eating fruit and vegetables, few are concerned about the potential health risks from chemical and microbiological contamination (*safe*food, 2007). According to the FAO/WHO, leafy greens, including fresh herbs, present the greatest concern in terms of microbiological hazards associated with fresh produce (FAO and WHO, 2008). In the EU, most cases of illness originating from foods of non-animal origin are caused by *Salmonella* in leafy greens eaten raw as salads (EFSA, 2013; Da Silva Felicio *et al*., 2015).

Outbreaks

Fresh herbs and salad leaves can become contaminated with pathogens from a number of sources, including:

1. Contaminated water used for irrigation or to apply pesticides, fungicides or plant feed
2. Human or animal sewage used as fertiliser
3. Contaminated water used to wash the crop after harvesting
4. Domestic or wild animal contact with the crop
5. Contaminated equipment used to wash, process or package the crop
6. Contaminated vehicles, crates or storage areas used in distribution
7. Cross-contamination during food preparation in other food businesses or in the home
8. Infected food handlers

Eating fresh herbs or salad leaves contaminated with pathogens has been reported as the cause of many outbreaks (Table 1). Depending on the distribution network, outbreaks associated with salad leaves and fresh herbs can be regional, national or international. As fresh herbs and salad leaves have a limited shelf-life, contaminated product may no longer be on the market by the time an outbreak comes to the attention of public health authorities. This explains why microbiological testing of fresh produce during outbreak investigations are often negative, and why outbreak investigations linked to fresh produce often rely on trace-back studies. This emphasises the importance of robust traceability[2] documentation by food business operators as required under Regulation (EC) No 178/2002 (European Commission, 2002). In addition, fresh herbs and salad leaves can be overlooked as the cause of an outbreak since they are often used as a flavouring or garnish, meaning that people who are ill do not remember eating them.

[1] (i) Public Health Laboratory, Limerick; (ii) Public Health Laboratory, Sligo General Hospital, Sligo; (iii) Public Health Laboratory, Waterford Regional Hospital, Waterford; (iv) Public Analyst`s Laboratory, Sir Patrick Duns Hospital, Grand Canal Street, Dublin; (v) Public Health Microbiology Laboratory, St Finbarr's Hospital, Cork; (vi) Public Health Microbiology Laboratory, Cherry Orchard Hospital, Dublin; and (vii) Public Health Microbiology Laboratory, Galway University Hospitals

[2] See also the FSAI's Guidance Note on Product Recall and Traceability (FSAI, 2013)

Ready-to-eat status

Salad leaves are usually eaten raw; and while fresh herbs are sometimes cooked, they are often consumed without cooking or added to food after or near the end of cooking, so that they don't receive sufficient heat treatment to kill pathogens. A cryptosporidiosis outbreak involving 23 guests at a wedding reception was believed to be caused by contaminated parsley which was added to béarnaise sauce after heating (Insulander *et al*., 2008). An outbreak of enterotoxigenic *E. coli* O78 in Norway, which made over 300 people ill, was believed to be caused by chives added to scrambled egg after cooking – the warm scrambled egg, served at a hotel buffet, provided a good environment for the *E. coli* O78 introduced by the chives to multiply (MacDonald *et al*., 2015).

If fresh herbs or salad leaves become contaminated with pathogens, they cannot be eliminated by washing, even with the use of sanitisers. Pathogens can adhere to plant surfaces, or become internalised through the stomata, cut edges of leaves or through the roots via contaminated irrigation water. If pathogens become internalised, they will not be affected by surface washing or disinfectant (Lynch *et al*., 2009). Using differential staining of living and dead cells, Seo *et al* (1999) showed that while *E. coli* O157 cells on the surface of iceberg lettuce leaves were killed by a 20 mg/L chlorine decontamination treatment, cells inside the stomata or those which had penetrated the cut edge of a leaf survived. Therefore, salad leaves and fresh herbs – including those labelled with the instruction to wash before eating – are considered ready-to-eat[3] (Lynch *et al*., 2009).

Prevalence of pathogens

The FSAI has not previously carried out a survey which specifically investigated the microbiological safety of fresh salad leaves and fresh herbs. However, a 2002 survey of the bacteriological safety of pre-cut fruit and vegetables, sprouted seeds and unpasteurised fruit and vegetable juices from processing and retail premises, detected *Salmonella* in one sample (0.2%; 1/529) – this was a bag of fresh spinach leaves contaminated with *Salmonella* Diarizonae (FSAI, 2002). While *L. monocytogenes* numbers in that survey were below the limit of enumeration[4] for 99% (342/344) of samples, one sample contained *L. monocytogenes* at between 20 and 100 cfu/g and another sample contained *L. monocytogenes* at 160 cfu/g, which exceeds the maximum legal limit of 100 cfu/g for *L. monocytogenes* in ready-to-eat foods (European Commission, 2005).

Prevalence studies in other countries have also found low level contamination of fresh herbs and salad leaves with pathogens (Table 2). In 2013, 77 notifications about microbiological contamination of fresh herbs and salad leaves were issued through the EU Rapid Alert System for Food and Feed (RASFF), the majority (55%) of which related to *Salmonella* in paan (betel) leaves (Appendix 1).

[3] Commission Regulation (EC) No 2073/2005 defines ready-to-eat food as "food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern"

[4] <20 cfu/g for the 2003 survey

Table 1: Selection of outbreaks associated with fresh herbs and salad leaves

Herb or salad leaf	Pathogen	Year	Country	Reference
Basil	*Salmonella* Senftenberg	2007	International: UK, Denmark, the Netherlands, USA	(Pezzoli *et al*., 2007), (Elviss *et al*., 2009)
Basil	*Shigella sonnei*	2011	Norway	(Guzman-Herrador *et al*., 2011), (Guzman-Herrador *et al*., 2013)
Basil	Enterotoxigenic *E. coli* and *Salmonella* Anatum	2006	Denmark	(Pakalniskiene *et al*., 2009)
Thai basil	*Cyclospora cayetanesis*	2001	Canada	(Hoang *et al*., 2005)
Chives	Enterotoxigenic *E. coli* (*E. coli* O78)	2012	Norway	(MacDonald *et al*., 2015)
Coriander	*Salmonella* Thompson	1999	USA	(Vernon Campbell *et al*., 2001)
Coriander	*E. coli* O157	2007	UK	(Whittaker *et al*., 2009)
Parsley	*Shigella sonnei* and enterotoxigenic *E. coli*	1998	USA and Canada	(Naimi *et al*., 2003)
Parsley	*Cryptosporidium*	2008	Sweden	(Insulander *et al*., 2008)
Curry leaves	*Salmonella* Agona	2013	UK	(Foster, 2013)
Iceberg lettuce	*Salmonella* Braenderup	2003	UK	(Gajraj *et al*., 2012)
Iceberg lettuce	*E. coli* O157	2005	Sweden	(Soderstrom *et al*., 2008)
Iceberg lettuce	*Salmonella* Newport and *Salmonella* Reading	2008	Finland	(Lienemann *et al*., 2011)
Iceberg and romaine lettuce	*E. coli* O157	2012	Canada	(Tataryn *et al*., 2014)
Lollo bionda lettuce	Norovirus and enterotoxigenic *E. coli*	2010	Denmark	(Ethelberg *et al*., 2010)
Romaine lettuce	*E. coli* O157	2011	USA	(Slayton *et al*., 2013)
Romaine lettuce	*E. coli* O145	2010	USA	(Taylor *et al*., 2013)
Rocket	Hepatitis A	2001	Sweden	(Nygård *et al*., 2001)
Rucola[(a)]	*Salmonella* Thompson	2004	Norway (and probably international)	(Nygård *et al*., 2008)
Spinach	*E. coli* O157	2006	USA	(Grant *et al*., 2008)
Watercress	*E. coli* O157	2013	UK	(Launders *et al*., 2013)
Lettuce	*Salmonella* Typhimurium DT104	2000	UK	(Horby *et al*., 2003)
Four leaf salad	*Salmonella* Newport	2001	UK	(Fisher and O'Brien, 2001)
Mixed salad leaves, containing mixed lettuce, dill, chives, parsley and green onions	*Cyclospora*	2000	Germany	(Döller *et al*., 2002)
Mixed salad containing radicchio rosso	*Yersinia enterocolitica*	2011	Norway	(MacDonald *et al*., 2011)
Mixed salad containing romaine, iceberg lettuce, cabbage and carrots	*E. coli* O157		USA	(Marder *et al*., 2014)

[(a)] Rucola is also known as rocket or arugula

Table 2: Prevalence of pathogens in fresh herbs and salad leaves

Produce type	Pathogen	% positive	Positive/number tested	Country	Reference
Lettuce	*Salmonella* spp.	0.7	1/137	Spain	(Sospedra *et al*., 2013)
	L. monocytogenes	0	0/137		
Lettuce and salad leaves	*L. monocytogenes*	0	0/35	Croatia	(Kovacevic *et al*., 2013)
Fresh herbs (pre-cut)	*Salmonella*	0.6	18[(a)]/3,018	UK	(Elviss *et al*., 2009)
Fresh herbs (growing in pots)		0	0/742		
Lettuce	*Salmonella*	0.2	1/530	Canada	(Arthur *et al*., 2007)
	Shigella	0	0/530		
	VTEC	0	0/530		
Fresh herbs	*Salmonella*	0	0/188		
	Shigella	0	0/188		
	VTEC	0	0/188		
Fresh herbs	*Salmonella*	0	0/184	USA	(Johnston *et al*., 2005)
	L. monocytogenes	0	0/184		
	E. coli O157	0	0/184		
Leafy greens	*Salmonella*	0	0/124		
	L. monocytogenes	0	0/124		
	E. coli O157	0	0/124		
Lettuce	*Salmonella*	0	0/200	Norway	(Johannessen *et al*., 2002)
	L. monocytogenes	0.5	1/200		
Pre-cut salad	*Salmonella*	0	0/100		
	L. monocytogenes	0	0/100		
Herbs growing in pots	*Salmonella*	0	0/130		
	L. monocytogenes	0	0/130		
Fresh herbs	*Salmonella*	0	0/100		
	L. monocytogenes	0	0/100		
Bagged salad vegetables (retail)	*Salmonella*	0.1	5/3,843	UK	(Sagoo *et al*., 2001a)
	L. monocytogenes	2.3	90[(b)]/3,849		
	Campylobacter	0	0/3,827		
	E. coli O157	0	0/3,820		
Organic cress, lettuce, watercress	*Salmonella*	0	0/492	UK	(Sagoo *et al*., 2001b)
	L. monocytogenes	0	0/492		
	Campylobacter	0	0/492		
	E. coli O157	0	0/492		

(a) The *Salmonella* positive samples were: basil (1.3%, 9/674); coriander (0.4%, 3/733); mint (0.3%, 1/397); parsley (0.3%, 2/774); other (0.6%, 3/487 – positive samples were curry leaves, 2 and walleria,1)

(b) *L. monocytogenes* was detected at >100 cfu/g (i.e. 660 cfu/g) in one of these 90 samples, the remainder were <20 cfu/g

AIM OF SURVEY

The aim of this survey was to investigate the microbiological safety of ready-to-eat, pre-cut and pre-packaged fresh herbs and salad leaves sampled from retail establishments in Ireland.

METHOD

Sample collection

Between 17 June and 31 October 2013 (inclusive), EHOs collected single samples (n=1) of ready-to-eat, pre-cut and pre-packaged fresh herbs and salad leaves from retail establishments such as supermarkets, convenience stores, green-grocers and market stalls. All samples were fully enclosed in sealed packaging, so that in the event of an unsatisfactory result, it would be clear that the product was contaminated at the production/packaging stage and not during distribution or retail. Samples were considered ready-to-eat unless they were specifically labelled with the instruction that they must be cooked before eating. Fresh herbs and salad labelled to indicate that they must be washed before eating are considered ready-to-eat.

Whole heads of lettuce and herbs growing in pots were excluded from this survey. Also excluded were salad leaves that included other ingredients, for example meat, fish, cheese, tomato, onion, beetroot. However, bagged salad leaves that contained additional ingredients, such as croutons or salad dressing, in a separate, sealed pouch were included.

Survey questionnaire

EHOs were asked to complete a survey questionnaire for each sample collected (Appendix 2). If samples were stored or displayed under refrigeration at the time of collection, EHOs were also asked to measure the air temperature of the refrigeration unit and record this on the laboratory sample submission form.

Sample analysis

Samples were analysed for the presence or absence of *Salmonella* spp. using EN/ISO 6579 and *L. monocytogenes* was enumerated using EN/ISO 11290-2. In addition, depending sufficient sample volume, three of the seven laboratories analysed samples they received for the presence or absence of VTEC (CEN/ISO TS 13136), *E. coli* O157 (ISO 16654:2001) and/or *E. coli* O26 ISO 16654:2001 modified).

Interpretation of results

Salmonella and *L. monocytogenes* results were compared against limits set in Commission Regulation (EC) No 2073/2005, as amended (European Commission, 2005):

- *Salmonella*: absence in 25 g[5]
- *L. monocytogenes*: 100 cfu/g[6]

As no legal microbiological criteria are set for VTEC in fresh herbs and salad leaves, the guideline limit of absence in 25 g was applied as outlined in the FSAI's Guidance Note No.3 (FSAI, 2001b).

[5] Pre-cut fruit and vegetables (ready-to-eat) (food category 1.19)
[6] Maximum legal limit for *L. monocytogenes* in ready-to-eat foods placed on the market during their shelf-life (food categories 1.2 and 1.3)

RESULTS AND DISCUSSION

Of the 1,005 samples included in this survey, 79% (n=795) were fresh salad leaves, 20% (n=202) were fresh herbs and eight samples (1%) were bags of salad leaves containing fresh herbs.

Microbiological results

The results of microbiological testing are presented in Table 3.

Table 3: Microbiological results

Parameter	Number of samples tested	Number (%) satisfactory	Number (%) unsatisfactory
Salmonella spp.	1,005	1,004 (99.9)	1 (0.1) [a]
L. monocytogenes (enumeration)	1,000[b]	1,000 (100)[c]	0
VTEC	247	247 (100)	0
E. coli O157 [d]	403	403 (100)	0
E. coli O26	397	397 (100)	0

[a] *S.* Napoli was detected in one bag of rocket leaves
[b] For five samples, there was insufficient sample to test for both *Salmonella* spp. and *L. monocytogenes*
[c] *L. monocytogenes* count was below the limit of enumeration (<10 cfu/g) for 99.8% (998/1,000) samples and 10 cfu/g for two samples (0.2%)
[d] *E. coli* O157 was detected in one bag of mixed spinach and watercress but the isolate was not of clinical significance as it did not contain the genes necessary to produce verotoxin (shiga toxin)

Salmonella

Salmonella was detected in 0.1% (1/1,005) samples tested, a similar prevalence level to that found in other studies (Table 2). The unsatisfactory sample was a bag of rocket leaves grown in Italy, from which *Salmonella* Napoli was isolated. As fresh salad leaves have a limited shelf-life, the 'use-by' date of the batch of rocket had passed by the time the *Salmonella* positive result was known[7]. A product withdrawal/recall[8] was not possible since the contaminated batch was no longer on the market, but the FSAI issued an alert through RASFF[9] to inform other Member States about the contamination and to provide the supplier's details to the Italian competent authority so that they could carry out follow-up investigations.

As a matter of routine, clinical laboratories in Ireland send *Salmonella* isolated from human cases of infection to the NSSLRL for serotyping and molecular subtyping. Typing allows the differentiation between strains of the same pathogen and can provide information on sources of contamination or illness, or help identify diffuse outbreaks of illness that may otherwise go undetected. For example, the molecular subtyping work carried out by the NSSLRL identified a national outbreak of salmonellosis linked to duck eggs (FSAI, 2010).

This is the first time *S*. Napoli has been isolated from food sampled in Ireland as part of official controls. There were no *S.* Napoli infections in humans reported in Ireland during June/July 2013 – the period corresponding to when the contaminated batch of rocket was on the market and the incubation period for *Salmonella* infection (12-

[7] Using the ISO reference method, time to a negative result is 72 hours, time to positive result is at least 120 hours (five days)
[8] Guidance Note No. 10: Product Recall and Traceability (Revision 3) (FSAI, 2013)
[9] 2013.0939: https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationDetail&NOTIF_REFERENCE=2013.0939

72 hours). Two cases of *S.* Napoli infection were reported in October 2013, but one of these cases was infected in Portugal. Indeed *S.* Napoli is a rare cause of salmonellosis in Ireland. Only 0.3% (13/4,952) of the human *Salmonella* isolates serotyped by the NSSLRL from 2002 to the end of 2014 was *S*. Napoli. Of those 13 cases, four were believed to have acquired the infection in another country, namely: France, Italy, Spain and Portugal (NSSLRL personal communication). Similarly in the EU as a whole, *S*. Napoli was responsible for only 0.6% (434/73,627) of human salmonellosis cases in 2013 (EFSA & ECDC, 2015).

The pulsed-field gel electrophoresis (PFGE) profile of the rocket isolate was very similar to that from a number[10] of human cases reported as acquired in Ireland or elsewhere in Europe in recent years (Appendix 3). Of particular interest is the similarity to isolates from a Swedish outbreak linked to eating rucola (rocket) salad imported from Italy (NSSLRL personal communication). This raises the possibility of some source of low level or intermittent contamination of salad vegetables with *S*. Napoli in Italy. In May 2013, Denmark also issued a RASFF (2013.0644) on *S.* Napoli in radicchio lettuce from Italy (Appendix 1).

Listeria monocytogenes

It is generally accepted that consuming *L. monocytogenes* at levels below 100 cfu/g food is of low risk to consumers (European Commission, 1999). Therefore, Commission Regulation (EC) No 2073/2005, as amended, sets a maximum legal limit of 100 cfu/g *L. monocytogenes* for ready-to-eat foods during their shelf-life[11].

In this survey, the *L. monocytogenes* count was below the limit of enumeration (<10 cfu/g) for 99.8% (998/1,000) of samples tested. For one bag of rocket and one bag of mixed spinach, watercress and rocket leaves, *L. monocytogenes* was enumerated at 10 cfu/g, well below the 100 cfu/g maximum legal limit set in Commission Regulation (EC) No 2073/2005. Again, the 'use-by' date of both bags of salad had passed by the time the enumeration result[12] was received.

The serotypes isolated from these two samples were 1/2a and 1/2c. In Ireland, serogroup 4b caused 64% (41/64) of the human listeriosis cases from 2005 to 2013, followed by 1/2a (27%), 1/2c (6%) and 1/2b (3%) (NSSLRL, 2013). In the EU, serotypes 1/2a and 4b also caused most of the 1,771 cases of human listeriosis in 2013: 1/2a (57.5 %), 4b (34.3 %), 1/2b (6.4 %), 1/2c (1.4 %), 3a and 3b (both 0.2 %) (EFSA & ECDC, 2015).

Verotoxigenic E. coli

Three laboratories tested samples specifically for *E. coli* O157 (n=403) and for *E. coli* O26 (n=397). No sample was positive for *E. coli* O26, and although *E. coli* O157 was detected in one bag of mixed salad leaves, the isolate did not contain the genes required to produce verotoxin (shiga toxin) and therefore, was not of clinical significance. Two laboratories used the CEN/ISO TS13136 method which targets the major VTEC virulence genes *stx* and *eae*. All 247 samples tested were negative.

Storage or display temperature

For fresh-cut leafy greens, proper temperature control of storage and transportation is critical to maintaining visual quality, crispiness and to delay microbial growth during the shelf-life (EFSA, 2014). Information on the temperature that samples were stored or displayed at the time the sample was collected was provided by the EHO on the laboratory sample submission form for 766/1,005 samples. Of these, 87% (n=663) were stored under refrigerated conditions and 13% (n=97) were at ambient temperature. The air temperature of the refrigeration unit was measured and recorded by EHOs for 438 of the refrigerated samples. For 77% (336/438) of these samples, the air temperature of the refrigeration unit was at the recommended ≤5°C[13], but was >5°C[14] for 23% (102/438) of samples.

[10] Six cases: 2013 (1), 2012 (1), 2011 (2), 2009 (1) and 2008 (1)
[11] Annex I, Chapter 1, food safety criteria, food categories 1.2/1.3 (European Commission, 2005)
[12] Using the ISO reference method, it takes 48 hours for a clear negative result and at least 120 hours (5 days) for a confirmed result
[13] Temperature range was -1.7 to 5°C; mean 3.4°C; median 4.0°C; mode 4.0°C
[14] Temperature range was 5.1 to 18°C; mean 7.8°C; median 6.5°C; mode 6.0°C

For the bag of rocket in which *Salmonella* was detected, the air temperature of the refrigeration unit was measured as 7.1ºC, which is above the maximum recommended refrigerated storage temperature of 5ºC. Chilled storage is an important control in maintaining the safety and quality of bagged salad leaves. *Salmonella* can grow at 7ºC and so the numbers of *Salmonella* on the already contaminated product could increase during the shelf-life, if sufficiently long. Sant'Ana *et al* (2012) demonstrated that both *Salmonella* and *L. monocytogenes* were able to grow in arugula (rocket) at 7ºC during its six day shelf-life, and that the growth potential increased if the product was temperature abused (stored at 15ºC) for part, or all of the shelf-life. Food business operators should ensure that refrigeration units do not exceed the maximum chilled temperature of 5ºC.

Information collected on questionnaire

EHOs were asked to complete a survey questionnaire (Appendix 2) to provide information about the sample that would not be captured on the usual laboratory sample submission form. A questionnaire was completed for 89% (893/1,005) of samples. The remainder of this section will discuss the questionnaire results.

Sample type

The percentage breakdown by sample type was the same for the questionnaire samples (n=893) as for the overall samples (n=1,005): 79% (n=703) were salad leaves, 20% (n=182) were herbs and 1% (n=8) were bags of salad leaves containing herbs.

Country of origin

As the Irish climate does not permit year-round growth of fresh herbs and salad leaves, at certain times of the year imported produce is more likely to be on the market. This survey was conducted between June and October, the months when Irish grown fresh herbs and salad leaves are most likely on the market (Bord Bia, 2013).

The country of origin was labelled on the packaging for 85% (756/893) of samples, with Ireland the most frequently cited country followed by the UK (Figure 1). None of the Irish-grown samples, which made up the majority (62%) of samples in this survey, were unsatisfactory.

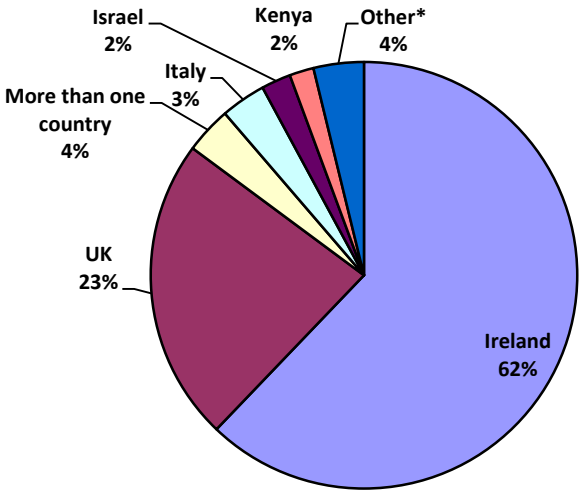

Figure 1: Country of origin, where stated (n=756)
*Other: The Netherlands 11, Spain 10, France 4, Morocco 2, Portugal 1 and Thailand 1

Washing instructions

Overall, the majority (67%; 598/893) of samples for which a questionnaire was returned were labelled to indicate that the product was already washed, while 18% (n=161) provided instructions that the product should be washed before eating. For 15% (n=134) of samples, there was no indication as to whether the product had been previously washed or whether it required washing before consumption. Consumers were more likely to be instructed to wash herbs before eating than salad leaves: 58% (105/182) of herb samples were labelled with this instruction, compared to 18% (161/893) of salad leaves.

In this survey, the bag of rocket leaves in which *Salmonella* was detected was already washed. Washing fresh herbs and salad leaves cannot eliminate pathogens if the product is contaminated. Even if sanitisers are used in the wash water they will have no effect on pathogens that have been internalised. Goldberg *et al* (2011) showed that the iceberg and arugula (rocket) leaves displayed the highest incidence of *Salmonella* internalisation through the stomata. A case-control study conducted for an outbreak of *E. coli* O157:H7 illness associated with spinach, which made 205 people ill, found that washing spinach before consumption did not affect the odds of falling ill (Grant *et al*., 2008). Bacteria are also more likely to adhere to the cut surface of plants where cell damage releases extra nutrients enabling bacteria counts to increase. A UK survey of fresh herbs at retail found that pre-cut herbs contained over twice as much *E. coli* at ≥100 cfu/g than those sold growing in open containers (Elviss *et al*., 2009).

Because washing won't eliminate pathogens, producers need to control potential points of contamination in the field, during harvesting, processing and distribution. They can achieve this by following guides to good agricultural and hygiene practice such as the FSAI's Code of Practice 4 (FSAI, 2001a[15]).

Storage instructions

So that food can be stored appropriately, any special storage conditions should be indicated on the label of pre-packaged food[16]. Clear information (including labelling) should be provided to consumers on appropriate handling of leafy greens which includes specific directions for product storage, preparation, intended use, 'use-by' date or other shelf-life indicators (EFSA, 2014).

In this survey, storage instructions were labelled on 76% (680/893) of samples for which a questionnaire was received. 97% (662/680) of the storage instructions indicated that the fresh herbs or salad leaves should be refrigerated. Refrigeration slows down the growth of bacteria helping to maintain safety and quality of the product. For ten samples (nine basil samples and one sample of thyme) the labelling indicated that the product should be kept at ambient temperature (Figure 2). Basil leaves are susceptible to chill damage and can blacken in the fridge; therefore, producers tend to advise that basil shouldn't be refrigerated. However, producers should be aware that a UK survey found that *Salmonella* was only detected in fresh herbs that were displayed at temperatures above 8ºC[17] (Elviss *et al*., 2009). This emphasises the need to control potential points of contamination in the field, during harvesting, processing and distribution.

Investigations of outbreaks often highlight poor temperature control and storage of cut herbs at room temperature. Chopping large volumes of contaminated parsley and holding at room temperature to use as a garnish, was believed to contribute to a large restaurant-associated outbreak of shigellosis since *Shigella* can grow rapidly on chopped parsley held at room temperature (Naimi *et al*., 2003). An investigation into a large outbreak of salmonellosis linked to chutney made from contaminated raw curry leaves found that the growth rate of *Salmonella* was likely increased when batches of the chutney were held at ambient temperature for some time on food stalls at an outdoor food event (Foster, 2013).

[15] Code of Practice 4 is being updated by the FSAI in 2015
[16] At the time of the survey this was a requirement under the general labelling rules (Directive 2000/13/EC) which has since been replaced by Regulation (EU) No 1169/2011 on the provision of food information to consumers (European Commission, 2011)
[17] Temperature range 10.3 to 22ºC

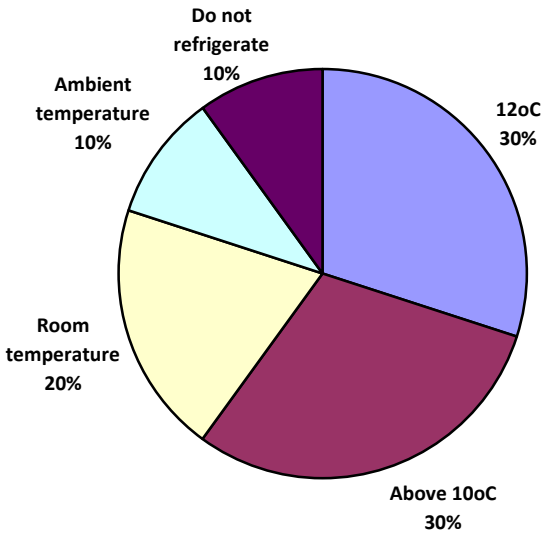

Figure 2: Range of ambient temperature storage instructions given (n=10)

Also noted in this survey was the wide range of storage instructions given regarding the temperature at which samples requiring refrigeration should be stored (Figure 3). Some advised a maximum storage temperature of 3ºC; however, the national standard for refrigerated storage at retail/wholesale level sets a maximum temperature of 5ºC (NSAI, 2007). Food business operators that package ready-to-eat, pre-cut, fresh herbs and salad leaves should also be aware that, when deciding on storage instructions and determining the shelf-life of their products, the temperature in domestic fridges is generally higher than at retail and wholesale level. A survey which monitored the temperature profile of 100 refrigerators in domestic kitchens in Ireland over 72 hours, found that 59% had an average temperature higher than 5ºC and 6% had an average temperature higher than 10ºC (Kennedy *et al*., 2005).

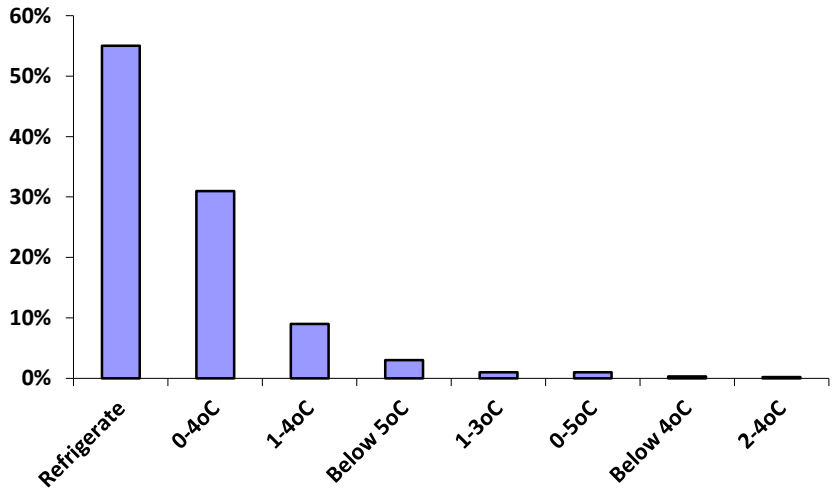

Figure 3: Range of chilled temperature storage instructions given (n=662)

RECOMMENDATIONS

From the results of this survey, the following recommendations are made:

- Food business operators that grow fresh, ready-to-eat herbs and salad leaves should take all reasonable measures to control potential points of contamination in the field, during harvesting, processing and distribution. This can be achieved by following guides to good practice, such as the FSAI's Code of Practice for Food Safety in the Fresh Produce Supply Chain in Ireland (FSAI, 2001a)

- Food business operators should ensure that refrigeration units do not exceed the maximum chilled temperature of 5^{o}C

- Food business operators that package fresh, ready-to-eat herbs and salad leaves should be aware, when determining shelf-life and deciding on storage instructions, that temperature in domestic fridges is generally higher than at retail and wholesale level

- Food business operators should ensure that their traceability records for fresh herbs and salad leaves are robust, as per Regulation (EC) No 178/2002 (European Commission, 2002). Following the procedures in the FSAI's Guidance Note No.10 on Product Recall and Traceability (FSAI, 2013) will facilitate rapid control measures to be implemented should a pathogen be detected in a batch of fresh herbs or salad leaves or if they are implicated in an outbreak of illness

- Food laboratories, both official and private, should send pathogens isolated from fresh herbs and salad leaves to the relevant reference laboratory for subtyping. This will enhance Ireland's information on sources of pathogens and provide valuable information when foodborne outbreaks are being investigated

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APPENDIX 1: 2013 RASFF NOTIFICATIONS FOR MICROBIOLOGICAL CONTAMINATION OF FRESH HERBS AND SALAD LEAVES

(Source RASFF portal: https://webgate.ec.europa.eu/rasff-window/portal/

No.	RASFF type	Notification date	RASFF reference	Notifying country	Subject
1	information for attention	02/01/2013	2013.0001	Denmark	*Salmonella* Bongori (present /25g) in lollo bionda lettuce from Germany
2	information for attention	21/01/2013	2013.0068	UK	*Salmonella* spp. (presence /25g) in lemon thyme from Morocco
3	information for attention	19/02/2013	2013.0211	UK	*Salmonella* spp. (presence /25g) in paan leaves (Betel) from India
4	information for attention	20/02/2013	2013.0216	UK	*Salmonella* spp. (presence /25g) in paan leaves from India
5	information for attention	20/02/2013	2013.0218	Norway	High count of *Escherichia coli* (810; 1500 CFU/g) in fresh basil leaves from Vietnam
6	information for attention	25/02/2013	2013.0248	UK	*Salmonella* (present /25g) in paan leaves (betel) from Bangladesh
7	information for attention	25/02/2013	2013.0247	UK	*Salmonella* (present /25g) in paan leaves (betel) from Bangladesh
8	border rejection	26/02/2013	2013.AOD	UK	*Salmonella* spp. (in 1 out of 5 samples /25g) in paan leaves (betel) from Bangladesh
9	border rejection	26/02/2013	2013.AOC	UK	*Salmonella* (presence /25g) in fresh paan leaves (betel) from Bangladesh
10	border rejection	07/03/2013	2013.APJ	UK	*Salmonella* spp. (presence /25g) in paan leaves (betel) from Bangladesh
11	border rejection	07/03/2013	2013.APM	UK	*Salmonella* spp. (present /25g) in paan leaves (betel/pan) from Bangladesh
12	border rejection	07/03/2013	2013.APL	UK	*Salmonella* spp. (presence /25g) in paan leaves (betel) from Bangladesh
13	border rejection	07/03/2013	2013.APK	UK	*Salmonella* spp. (present /25g) in paan leaves (betel/pan) from Bangladesh
14	border rejection	07/03/2013	2013.APH	UK	*Salmonella* spp. (presence /25g) in paan leaves (betel) from Bangladesh
15	information for attention	07/03/2013	2013.0313	UK	*Salmonella* in paan leaves (betel) from Bangladesh
16	information for attention	07/03/2013	2013.0312	UK	*Salmonella* spp. (present /25g) in paan leaves (betel/pan) from Bangladesh
17	information for attention	18/03/2013	2013.0384	UK	*Salmonella* spp. (in 2 out of 5 samples /25g) in paan leaves (Piper sarmentosum) from Cambodia
18	information for attention	18/03/2013	2013.038	UK	*Salmonella* spp. (presence /25g) in crested latesummer mint from Cambodia
19	information for attention	08/04/2013	2013.0493	Norway	High count of *Escherichia coli* (620<=>4400 CFU/g) in basil leaves from Cambodia
20	information for attention	12/04/2013	2013.0532	Norway	High count of *Escherichia coli* (30000/9000/24000/10000/33000 CFU/g) in fresh sweet basil from Cambodia

No.	RASFF type	Notification date	RASFF reference	Notifying country	Subject
21	information for attention	12/04/2013	2013.0528	Norway	High count of *Escherichia coli* (>15000; >15000; 800; >15000; >15000 CFU/g) in fresh sweet basil from Cambodia
22	information for attention	12/04/2013	2013.053	Norway	Too high count of *Escherichia coli* (7000/8000/10000/>150000/43000 CFU/g) in fresh sweet basil from Cambodia
23	information for attention	15/04/2013	2013.0541	UK	*Salmonella* spp. (present /25g) in paan leaves from Sri Lanka
24	information for attention	17/04/2013	2013.0553	Norway	High count of *Escherichia coli* (1200 CFU/g) in spinach from Cambodia
25	information for attention	19/04/2013	2013.0566	Norway	High count of *Escherichia coli* (between 130 and 980 CFU/g) in fresh peppermint from Cambodia
26	information for attention	19/04/2013	2013.0565	Norway	High count of *Escherichia coli* (between 250 and 6000 CFU/g) in fresh sweet basil from Cambodia
27	information for attention	02/05/2013	2013.0618	Norway	High count of *Escherichia coli* (10 / 300 / 540 / 30 / 220 CFU/g) in cockscomb mint from Vietnam
28	information for attention	02/05/2013	2013.0619	Norway	High count of *Escherichia coli* (30 / 2400 / 480 / 30 / 100 CFU/g) in fresh perilla from Vietnam
29	information for attention	02/05/2013	2013.0617	Norway	high count of *Escherichia coli* (2000; 2300; 2400; 2300 CFU/g) in fresh hary basil from Vietnam
30	information for attention	08/05/2013	2013.0644	Denmark	*Salmonella* Napoli (present /25g) in radicchio lettuce from Italy
31	information for attention	15/05/2013	2013.0675	Norway	*Salmonella* Weltevreden in fresh spinach from Sri Lanka
32	information for attention	15/05/2013	2013.0676	Norway	High count of *Escherichia coli* (26000 CFU/g) in centella (Centella asiatica) from Sri Lanka
33	border rejection	16/05/2013	2013.AYR	UK	*Salmonella* spp. (1 out of 5 samples /25g) in paan leaves (betel/pan) from Bangladesh
34	border rejection	16/05/2013	2013.AYT	Denmark	*Salmonella* (present /25g) in holy basil fresh leaves from Thailand
35	border rejection	22/05/2013	2013.AZN	UK	*Salmonella* spp. (presence /25g) in paan leaves from Thailand
36	information for attention	23/05/2013	2013.071	UK	*Salmonella* spp. in paan leaves from Thailand
37	information for attention	23/05/2013	2013.0709	UK	*Salmonella* spp. in paan leaves from Thailand
38	alert	27/05/2013	2013.0731	Norway	*Campylobacter* (presence /25g) in fresh dill from Italy
39	border rejection	30/05/2013	2013.BAV	Denmark	*Salmonella* spp. (presence /25g) in fresh sweet basil leaves from Thailand
40	border rejection	04/06/2013	2013.BBR	Finland	*Salmonella* spp. (presence /25g) in fresh curry leaves from Uganda

No.	RASFF type	Notification date	RASFF reference	Notifying country	Subject
41	border rejection	04/06/2013	2013.BBP	Finland	*Salmonella* Ituri in curry leaves from Uganda
42	border rejection	05/06/2013	2013.BBX	UK	*Salmonella* spp. (presence /25g) in paan leaves from Bangladesh
43	border rejection	07/06/2013	2013.BCL	Finland	*Salmonella* Infantis (presence /25g) in fresh sorrel leaves (Hibiscus sabdariffa) from India
44	information for attention	04/07/2013	2013.0939	Ireland	*Salmonella* Napoli (presence /25g) in wild rocket from Italy, via the United Kingdom
45	border rejection	16/07/2013	2013.BHT	UK	*Salmonella* spp. (in 2 out of 5 /25g) in paan leaves from India
46	information for attention	16/07/2013	2013.0997	UK	*Salmonella* spp. (presence /25g) in paan leaves from India
47	border rejection	09/08/2013	2013.BLQ	UK	*Salmonella* spp. (2 out of 5 samples /25g) in paan leaves (betel/pan) from India
48	information for attention	09/08/2013	2013.1113	UK	*Salmonella* spp. (5 out of 5 samples /25g) in paan leaves from Thailand
49	border rejection	14/08/2013	2013.BMJ	UK	*Salmonella* spp. (presence /25g) in paan leaves from India
50	information for attention	05/09/2013	2013.1214	UK	*Salmonella* spp. (in 1 out of 5 samples /25g) in paan leaves (betel/pan) from Thailand
51	border rejection	09/09/2013	2013.BOX	UK	*Salmonella* spp. in paan leaves (betel) from India
52	information for attention	10/09/2013	2013.1236	Norway	High count of *Escherichia coli* (1300; 70; 1100; 8; <10 cfu/g) in fresh spinach from Sri Lanka
53	alert	27/09/2013	2013.1314	Sweden	*Salmonella* Umbilo (presence /25g) in baby spinach and rucola salad with raw material from Italy, packaged in Sweden
54	border rejection	08/10/2013	2013.BSY	UK	*Salmonella* (in 2 out of 5 samples) in paan leaves from Bangladesh
55	border rejection	08/10/2013	2013.BSX	UK	*Salmonella* spp. (in 3 out of 5 samples /25g) in paan leaves from Bangladesh
56	border rejection	08/10/2013	2013.BSZ	UK	*Salmonella* (present /25g) in paan leaves from Bangladesh
57	border rejection	09/10/2013	2013.BTH	UK	*Salmonella* spp. (presence /25g) in paan leaves from Bangladesh
58	border rejection	09/10/2013	2013.BTG	UK	*Salmonella* spp. (in 2 out of 5 samples /25g) in paan leaves (betel/pan) from India
59	border rejection	09/10/2013	2013.BTC	UK	*Salmonella* spp. (in 1 out of 5 samples /25g) in paan leaves (betel/pan) from Bangladesh
60	information for attention	17/10/2013	2013.1378	Norway	*Salmonella* Lexington (present /25g) in sweet basil from Laos
61	border rejection	30/10/2013	2013.BWJ	Denmark	*Salmonella* Brunei (in 1 out of 5 samples /25g) in fresh coriander leaves from Thailand
62	border rejection	13/11/2013	2013.BYG	UK	*Salmonella* spp. in paan leaves from Bangladesh

No.	RASFF type	Notification date	RASFF reference	Notifying country	Subject
63	information for attention	18/11/2013	2013.1516	Norway	high count of *Escherichia coli* (1100 CFU/g) in fresh mint leaves from Cambodia
64	border rejection	22/11/2013	2013.BZP	UK	*Salmonella* spp. (presence /25g) in paan leaves from India
65	border rejection	22/11/2013	2013.BZO	UK	*Salmonella* spp. (presence /25g) in curry leaves from India
66	information for attention	25/11/2013	2013.1546	Denmark	high count of *Escherichia coli* (8600 CFU/g) in romaine lettuce from Spain
67	information for attention	29/11/2013	2013.1575	Finland	*Bacillus cereus* (17000 CFU/g) in packed rocket from the Netherlands
68	border rejection	29/11/2013	2013.CAL	Finland	*Salmonella* Weltevreden (present /25g) in fresh centella (Centella asiatica) from Sri Lanka
69	border rejection	02/12/2013	2013.CAQ	Finland	*Salmonella* Poona in fresh water spinach from Sri Lanka
70	information for attention	06/12/2013	2013.1624	UK	*Salmonella* spp. (presence /25g) in paan (or betel) leaves from India
71	information for attention	06/12/2013	2013.1623	UK	*Salmonella* spp. (presence /25g) in paan (or betel) leaves from India
72	border rejection	09/12/2013	2013.CBM	UK	*Salmonella* (presence /25g) in paan leaves from Bangladesh
73	border rejection	09/12/2013	2013.CBL	UK	*Salmonella* (present/25g) in paan leaves from India
74	border rejection	09/12/2013	2013.CBK	UK	*Salmonella* spp. (presence /25g) in paan leaves from Bangladesh
75	information for attention	11/12/2013	2013.1642	Sweden	*Salmonella* Szentes (present /25g) in rucola from Spain
76	border rejection	12/12/2013	2013.CCF	UK	*Salmonella* spp. in paan leaves from Bangladesh
77	border rejection	16/12/2013	2013.CDA	UK	*Salmonella* (present /25g) in paan leaves from India

APPENDIX 2: SURVEY QUESTIONNAIRE

1) **EHO's name** _____ 2) **EHO's sample Ref No.** _____ 3) **Lab Ref No** (see lab report) _____

Producer/packer/supplier details

4) Producer/packer/supplier name_____

5) Producer/packer/supplier contact details_____

Sample type

6) Was this sample: fresh herbs ☐, fresh salad leaves ☐ or both ☐?

7) Was the sample: one type of herb/salad leaf ☐ or mixed sample ☐

8) Herb name(s): basil ☐, chives ☐, coriander (cilantro) ☐, curry leaves ☐, dill ☐, marjoram ☐, mint ☐, oregano ☐, parsley ☐, rosemary ☐, sage ☐, tarragon ☐, thyme ☐, other ☐ (please specify) _____

9) Salad leaves name(s): cos (romaine) ☐, escarole ☐, frisee endive ☐, iceberg ☐, little gem ☐, lamb's lettuce ☐, lollo rosso ☐, mizune ☐, oak leaf lettuce ☐, rocket ☐, round lettuce ☐, radicchio ☐, red chard ☐, spinach ☐, watercress ☐, other ☐ (please specify) _____

Country of origin

10) Was the country where the sample was grown or packaged supplied? Yes ☐ (please specify) _____ **or** No ☐?

Date marking

11) Was a **use-by** date labelled? Yes ☐ (please specify) _____ **or** No ☐?

12) Was a **best-before** date labelled? Yes ☐ (please specify) _____ **or** No ☐?

13) Was a **display until** date labelled? Yes ☐ (please specify) _____ **or** No ☐?

Washing instructions

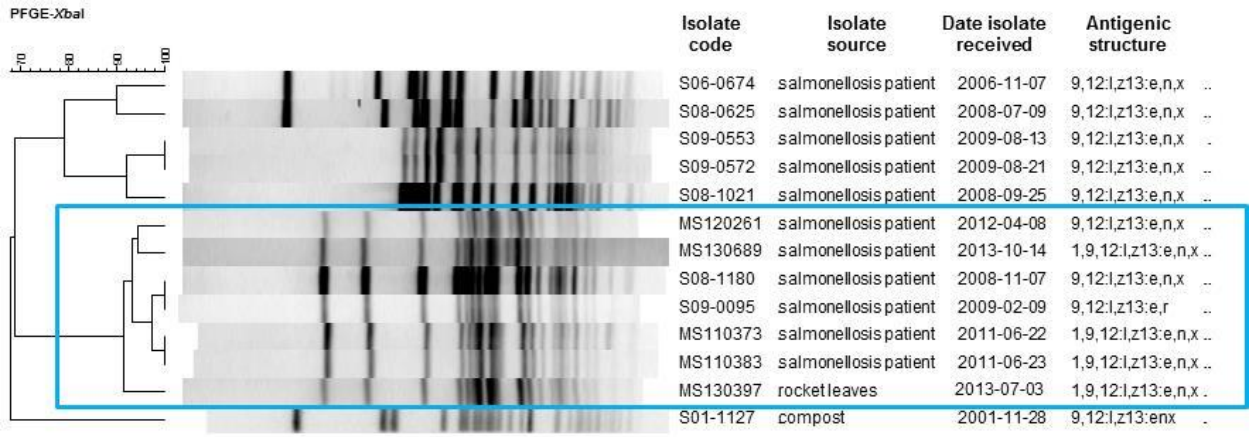
14) Was the sample labelled to indicate that it: was already washed ☐, must be washed before consumption ☐, **or** no washing instructions given ☐?

Storage instructions

15) Were storage instructions provided on the label? Yes ☐ (please specify **as written on label**) _____ ___ **or** No ☐?

End of survey, thank you

APPENDIX 3: DENDROGRAM SHOWING RELATEDNESS OF *S.* NAPOLI ISOLATES IN IRELAND



Isolate code	Isolate source	Date isolate received	Antigenic structure
S06-0674	salmonellosis patient	2006-11-07	9,12:l,z13:e,n,x ..
S08-0625	salmonellosis patient	2008-07-09	9,12:l,z13:e,n,x ..
S09-0553	salmonellosis patient	2009-08-13	9,12:l,z13:e,n,x .
S09-0572	salmonellosis patient	2009-08-21	9,12:l,z13:e,n,x ..
S08-1021	salmonellosis patient	2008-09-25	9,12:l,z13:e,n,x ..
MS120261	salmonellosis patient	2012-04-08	9,12:l,z13:e,n,x ..
MS130689	salmonellosis patient	2013-10-14	1,9,12:l,z13:e,n,x ..
S08-1180	salmonellosis patient	2008-11-07	9,12:l,z13:e,n,x ..
S09-0095	salmonellosis patient	2009-02-09	9,12:l,z13:e,r ..
MS110373	salmonellosis patient	2011-06-22	1,9,12:l,z13:e,n,x ..
MS110383	salmonellosis patient	2011-06-23	1,9,12:l,z13:e,n,x ..
MS130397	rocket leaves	2013-07-03	1,9,12:l,z13:e,n,x .
S01-1127	compost	2001-11-28	9,12:l,z13:enx .

Source: NSSLRL

Pulsed-field gel electrophoresis (PFGE) was carried out using *Xba*I restriction enzyme. The seven isolates highlighted are very similar. Six were from human salmonellosis cases and one isolate was from the *Salmonella*-positive bag of rocket found in this survey – however, none of the human cases of infection corresponded to the time period when the contaminated batch of rocket was on the market.

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