



Microbiological safety of pre-packaged sandwiches (09NS1)

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SUMMARY

This study investigated the microbiological safety of 948 pre-packaged sandwiches on sale in the Republic of Ireland. The survey revealed that 0.2% (2/948) of samples were categorised unacceptable/potentially hazardous for *Listeria monocytogenes* (>100 cfu/g) according to legal criteria and 0.3% (3/948) were unsatisfactory (≥ 100 but $< 10^4$ cfu/g) for coagulase positive staphylococci according to guideline criteria. This was a slight improvement on the results of a previous sandwich survey carried out by the Food Safety Authority of Ireland in 2002, where 0.3% (1/475) of samples were unacceptable/potentially hazardous for *L. monocytogenes* and 0.6% (3/475) were unsatisfactory for coagulase positive staphylococci¹³.

Of concern was the finding that 29% (228/782)^a of sandwiches were stored or displayed at temperatures higher than the recommended $\leq 5^\circ\text{C}$. In fact, four of the five sandwiches which were classified as unsatisfactory or unacceptable/potentially hazardous were stored above 8°C , with one sandwich displayed unrefrigerated at 17.9°C . For three of these sandwiches (all stored or displayed at $>8^\circ\text{C}$), there was at least one day remaining until the use-by date expired, which could allow the staphylococci or *L. monocytogenes* to multiply.

There was a high level of compliance (99%; 461/466) with the requirement for sandwiches that are delivered pre-packaged to be labelled with a use-by date. However, two food safety issues were noted: 1) the use-by date had expired for a small proportion (1%; 6/440) of samples collected and 2) there is the possibility that the shelf-life given to some sandwiches was too long.

The shelf-life of fresh or minimally processed foods can be extended by using modified atmosphere packaging (MAP), in which the oxygen content of the pack is reduced by flushing with gas, e.g. carbon dioxide and nitrogen. In this survey, only 1.3% (8/629) of sandwiches used MAP, indicating that this is not common practice for sandwiches sold in the Republic of Ireland.

From the results of this survey it is recommended that retailers ensure that sandwiches are stored or displayed at temperatures $\leq 5^\circ\text{C}$, and that they do not offer for sale sandwiches which have passed their use-by date. Manufacturers must ensure that accurate and realistic use-by dates are applied to the sandwiches they make.

^a Samples for which a storage temperature was given on the survey questionnaire

ACKNOWLEDGEMENTS

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ABBREVIATIONS

a_w	Water activity (a measure of water content)
cfu/g	Colony forming units per gram
EC	European Community
EU	European Union
EHO	Environmental health officer(s)
EFSA	European Food Safety Authority
EN	European standards
FSAI	Food Safety Authority of Ireland
HSE	Health Service Executive
ISO	International Organization for Standardization
n/a	Not applicable
p	p-value
µg	Microgram



INTRODUCTION

This survey examined the microbiological safety of pre-packaged sandwiches, available on sale in the Republic of Ireland, by testing for the pathogens *Listeria monocytogenes* and coagulase positive staphylococci.

L. monocytogenes infection in humans is mostly foodborne, resulting in febrile gastroenteritis or invasive systemic infection⁹. Febrile gastroenteritis occurs when otherwise healthy individuals consume high numbers (>8 log cfu) of *L. monocytogenes*. This form of listeriosis is usually self-limiting, although infection with highly virulent strains of serotype 4b can be fatal³⁰. The invasive form of the disease occurs mostly in people whose immune system is compromised (e.g. pregnant women and neonates, the young, older people, or those with chronic illness) and has a high fatality rate of up to 44%¹.

L. monocytogenes is of particular significance to food businesses producing ready-to-eat foods because of the bacterium's ability to establish itself, and persist, in the food processing environment. Also, because of its hardy nature, *L. monocytogenes* can survive and grow between 0.5°C and 45°C, at salt concentrations up to 20% weight /volume, and at low water activity (a_w 0.91)³⁰.

L. monocytogenes has been detected in 2.7-17% of pre-packaged sandwiches^{13,20,22,25,27}. Furthermore, listeriosis outbreaks have been associated with a range of ready-to-eat foods commonly used as ingredients in sandwiches, including deli meats, smoked salmon, coleslaw and soft cheese¹. Between 1999 and 2008, seven listeriosis outbreaks were potentially linked to sandwiches supplied to hospitals in the UK. The most recent of these was an outbreak of seven cases in Belfast, three of whom died²¹. In a Canadian outbreak in 2008, caused by the consumption of ready-to-eat meat products contaminated with *L. monocytogenes*; 57 people were made ill and 22 died¹⁹.

Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs sets a limit of 100 cfu/g for *L. monocytogenes* in ready-to-eat foods (other than those intended for infants and for special medical purposes) during their shelf-life¹¹. The British Sandwich Association recommends a target of <10 cfu/g of *L. monocytogenes* in sandwiches⁷. The Regulation also requires that food business operators manufacturing ready-to-eat foods, which may pose a *L. monocytogenes* risk for public health, sample processing areas and equipment for *L. monocytogenes* as part of their sampling scheme.

Staphylococcal foodborne disease results from the consumption of food contaminated with staphylococcal enterotoxin. The coagulase positive *Staphylococcus aureus* is responsible for the majority of cases of illness, although other coagulase positive staphylococci (namely, *S. intermedius* and *S. hyicus*) are also known to be able to produce enterotoxin⁴. As *S. aureus* can be carried in the nose, throat, under the fingernails, and in skin lesions, food handlers are an important source of food contamination^{8,28}. Although *S. aureus* is destroyed by cooking, the enterotoxin is heat stable and only small amounts ($\leq 1 \mu\text{g}$) are required to cause illness, which is characterised by a short incubation period (2-6 hours), nausea, vomiting, abdominal pain and diarrhoea⁵.

Time and temperature abuse of contaminated food is required for enterotoxins to form since *S. aureus* only produces enterotoxins once it reaches high levels (>10⁵ cfu/g)^{11,28}. In July 2009, 47 members of the UK police force suffered staphylococcal food poisoning after consuming sandwiches that had been stored, unrefrigerated, in a van³. Most strains of *S. aureus* grow between 7°C and 48°C with an optimum growth temperature of 35°C to 40°C⁴.

OBJECTIVES

- 1) To examine the microbiological safety of pre-packaged sandwiches with respect to *Listeria monocytogenes* and coagulase positive staphylococci^b.
- 2) Within the constraints of the study, to determine the effect of storage temperature, ingredients, packaging atmosphere etc. on microbiological safety.

^b Staphylococci are classified on their ability to produce coagulase, a blood clotting enzyme. *S. aureus* is coagulase positive. The method used in this survey (ISO 6888-1 or 2) is for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) from food and animal feeding stuffs

METHOD

This survey examined the microbiological safety of pre-packaged sandwiches, available on sale in the Republic of Ireland, by testing for the pathogens *Listeria monocytogenes* and coagulase positive staphylococci.

Sample Collection

From April to July, 2009 (inclusive) environmental health officers (EHOs) were requested to collect pre-packaged sandwiches from retail^c or service sector^d establishments across the Republic of Ireland. Samples were required to be a minimum of 100 g, but could include two pre-packaged sandwiches (e.g. 2 x 75 g sandwiches) provided they were from the same production batch and were clearly labelled for the laboratory. The temperature of the storage/display unit was measured at the time of sampling. EHOs were asked to complete a survey questionnaire (Annex 1) and to return it to the FSAI by 11th September 2009.

Only pre-packaged sandwiches were included in this survey, i.e. those that had been delivered to the premises pre-packaged or were pre-packaged on site. Excluded from the survey were sandwiches made to order, unwrapped sandwiches and sandwiches which received heat-treatment (e.g. toasted sandwiches and paninis). Pre-packaged sandwiches made from all types of bread were included and those with all types of packaging. In order to minimise over-sampling of major brands, EHOs were asked to submit only one sample of each sandwich type per brand, and to sample local brands where available.

Sample Analysis

All samples were stored and transported to the laboratory under appropriate conditions, i.e. in a cool box. Samples were tested for *L. monocytogenes* (ISO 11290-2) and coagulase positive staphylococci (ISO 6888-1 or 2). If coagulase positive staphylococci were present at levels $\geq 10^4$ cfu/g, laboratories were asked to test the sandwich for staphylococcal enterotoxin.

Classification of Results

Microbiological criteria for *L. monocytogenes* in ready-to-eat foods are specified in Commission Regulation (EC) No 2073/2005 (as amended) (Table 1). Microbiological criteria for coagulase positive staphylococci and staphylococcal enterotoxin in ready-to eat foods (other than cheeses, milk powder and whey powder) are not specified in the Regulation; however, criteria are specified in microbiological guidelines from the FSAI (Table 2).

Statistical Analysis

Chi square (χ^2) and Fisher's Exact Test analysis was performed using SPSS version 14.0 (with significance defined at the $p \leq 0.05$ level).

^c Retail: supermarkets, corner shops, farmers' markets, vending machines, forecourts, shops within healthcare settings etc.

^d Service sector: restaurants, hotels, cafés, take-aways, sandwich bars, cafés/restaurants/canteens in health care settings (e.g. hospitals, nursing homes) and sandwiches served to patients, etc.

Table 1: Microbiological criteria for *Listeria monocytogenes* in ready-to-eat food, as specified in Commission Regulation (EC) No 2073/2005

Food Category	Sampling Plan ^a		Limits ^b		Analytical reference method ^c	Stage where criterion applies
	n	c	m	M		
Ready-to-eat foods able to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes	5	0	100	cfu/g ^d	EN/ISO 11290-2 ^e	Products placed on the market during their shelf-life
	5	0	Absence	in 25 g ^f	EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
Ready-to-eat foods unable to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes ^g	5	0	100	cfu/g	EN/ISO 11290-2 ^e	Products placed on the market during their shelf-life

^a n=number of units comprising the sample; c=number of sample units giving values between m and M. These criteria are applicable to batch samples (n=5); however, for official sampling, single samples are permitted at retail level (single sampling is carried out in the context of a monitoring and surveillance programme)

^b In this case m=M

^c The most recent version of the standard should be used

^d This criterion shall apply if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life. The operator may fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life

^e 1 ml of inoculum is plated on a Petri dish of 140 mm diameter or on three Petri dishes of 90 mm diameter

^f This criterion shall apply to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life

^g Products with pH ≤ 4.4 or a_w ≤ 0.92, products with pH ≤ 5.0 and a_w ≤ 0.94, products with a shelf-life of less than five days shall be automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification



Table 2: Microbiological guidelines for coagulase positive staphylococci in ready-to-eat foods ^a

Food category	Microbiological safety (cfu/g)			
	Satisfactory	Acceptable	Unsatisfactory	Unacceptable/potentially hazardous
All ready-to-eat foods with the exception of: - cheese made from raw milk - cheeses made from milk that has undergone a lower heat treatment than pasteurisation and ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment -shelled and shucked products of cooked crustaceans and molluscan shellfish	<20	20 to <100	100 to <10 ⁴	≥ 10 ⁴ and staphylococcal enterotoxin present ^b

^a These limits (for *coagulase positive staphylococci* in ready-to-eat foods sampled at the point of sale) are adapted from the former FSAI Guidance Note Number 3. This Guidance Note is currently undergoing revision

^b If coagulase positive staphylococci are present at levels ≥ 10⁴ cfu/g, the sample should be tested for staphylococcal enterotoxin (i.e. the sample should be tested for the presence of the staphylococcal enterotoxin rather than the isolates' ability to produce toxin)



RESULTS AND DISCUSSION

Sample Collection

In total, 948 of the 971 samples collected by EHOs were considered for this report; 23 samples which were collected outside the specified time-frame (April – July) were excluded. The number of samples submitted from each HSE region is presented in Appendix 2.

Of the samples included, nearly two thirds (601/948) were collected in June and July (Figure 1). Almost 80% (740/948) of samples were collected from the retail sector (Figure 2).

Figure 1: Month samples were collected (n=948)

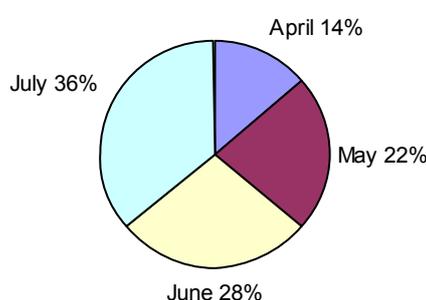
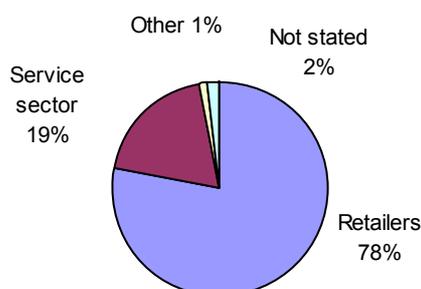


Figure 2: Food sector from which samples were collected (n=948)



Microbiological Safety

The results of this survey reveal over 99% of sandwiches tested were satisfactory for *L. monocytogenes* (Figure 3) and coagulase positive staphylococci (Figure 4). In all, two sandwiches were unacceptable/potentially hazardous for *L. monocytogenes* and three were unsatisfactory for coagulase positive staphylococci (Table 3). As no sample contained coagulase positive staphylococci at levels $\geq 10^4$ cfu/g, testing was not carried out for staphylococcal enterotoxin^e. The range of microbiological counts obtained is presented in Appendix 3.

^e Food is not considered unsafe until staphylococcal enterotoxins are produced. Enterotoxins are produced only once coagulase positive staphylococci reach levels of $>10^5$ cfu/g

Figure 3: Microbiological classification for *L. monocytogenes* for all samples (n=948)

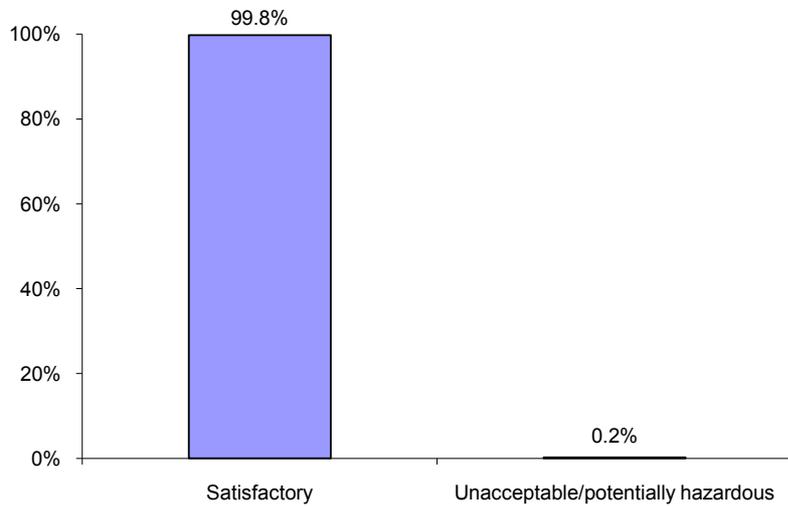


Figure 4: Microbiological classification for coagulase positive staphylococci for all samples (n=948)

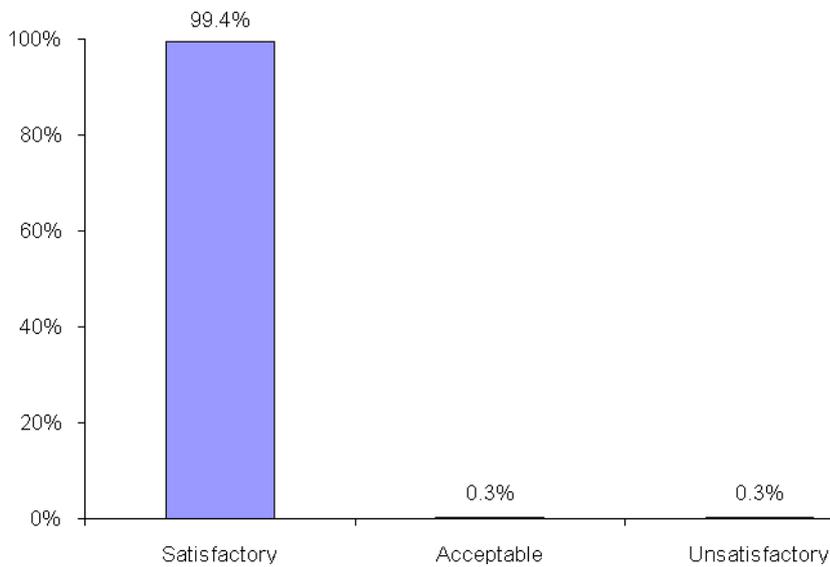


Table 3: Details of samples classified as unsatisfactory or unacceptable/potentially hazardous

<i>L. monocytogenes</i> count	430 cfu/g <u>unacceptable/ potentially hazardous</u>	2,900 cfu/g <u>unacceptable/ potentially hazardous</u>	<10 cfu/g satisfactory	<20 cfu/g satisfactory	<20 cfu/g satisfactory
Coagulase positive staphylococci count	<20 cfu/g satisfactory	<20 cfu/g satisfactory	120 cfu/g <u>unsatisfactory</u>	150 cfu/g <u>unsatisfactory</u>	310 cfu/g <u>unsatisfactory</u>
Description	Chicken and stuffing sandwich	Turkey salad sandwich	Chicken and bacon club sandwich	Chicken fajita sandwich	Egg salad sandwich
Month collected	June	May	July	July	June
Sample source	Forecourt	Forecourt	Café	Café	Corner shop
Storage/display conditions	8.8°C refrigerated	4.5°C refrigerated	10°C refrigerated	10°C refrigerated	17.9°C ambient
Packaging	Delivered pre-packaged	Pre-packaged on site	Delivered pre-packaged	Delivered pre-packaged	Pre-packaged on site
Days until use by date	2	n/a	1	1	n/a
Packaged in modified atmosphere?	No	No	No	No	No
Fillings	Chicken Stuffing Mayonnaise	Turkey Salad Mayonnaise	Chicken Pork Hard cheese	Chicken Salad Mayonnaise	Egg Salad

n/a: not applicable as the survey questionnaire only requested a use-by date for sandwiches which were delivered pre-packaged. The general labelling legislation does not require a use-by date to be labelled on foods which are pre-packaged on the premises from which they are sold

In the current survey, *L. monocytogenes* was detected at levels >100 cfu/g in 0.2% (2/948) of sandwiches. This is similar ($p>0.05$) to the findings of the national microbiological survey undertaken in 2002¹³, in which *L. monocytogenes* were detected at levels >100 cfu/g in 0.3% (1/323) of sandwiches. It is also similar ($p>0.05$) to the findings of UK studies where *L. monocytogenes* was detected at levels >100 cfu/g in 0.4% (1/1,088)²⁵ and 0.1% (1/1,538)²⁷ of sandwiches.

In the current survey, coagulase positive staphylococci were detected at unsatisfactory levels (≥ 100 but $<10^4$ cfu/g) in 0.3% (3/948) of sandwiches, which is similar to the findings of the 2002 national microbiological survey¹³, where coagulase positive staphylococci were detected at levels >100 cfu/g in 0.6% (3/475) of sandwiches. It is also similar to the findings of two recent UK surveys in which *S. aureus* was detected at levels >100 cfu/g in 0.5% (1/185)²⁶ and 0.6% (20/3249)²² of sandwiches. The results appeared to differ from those recorded by Little *et al.* in 2002, when 2% (10/449) of sandwiches were unsatisfactory for *S. aureus*²³.



Analysis of Questionnaires

The questionnaire return rate for this survey was 85% (802/948). As with the overall results, more than 99% of samples for which a questionnaire was returned were satisfactory for both *L. monocytogenes* and coagulase positive staphylococci (Figures 5&6). The microbiological results of these 802 samples are similar ($p>0.05$) to the microbiological results of the 948 samples presented in Figures 3&4; therefore, in terms of microbiology these 802 samples are representative of the total sample population.

Figure 5: Microbiological classification for *L. monocytogenes* for samples for which a questionnaire was returned (n=802)

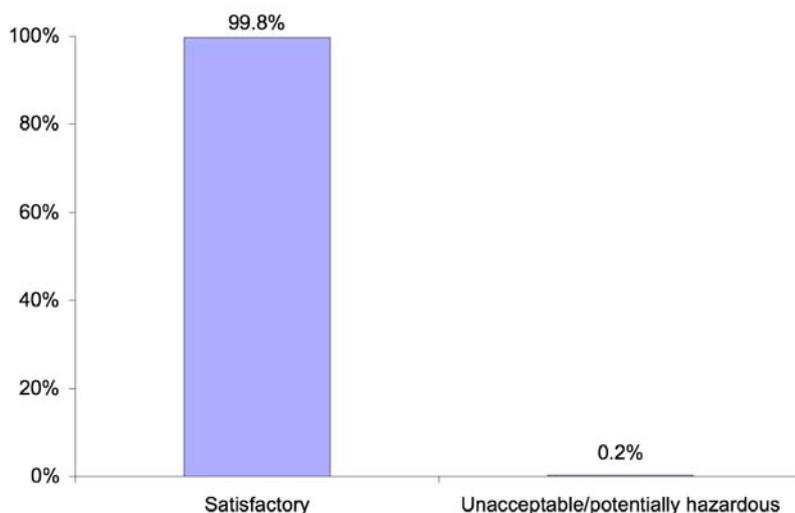
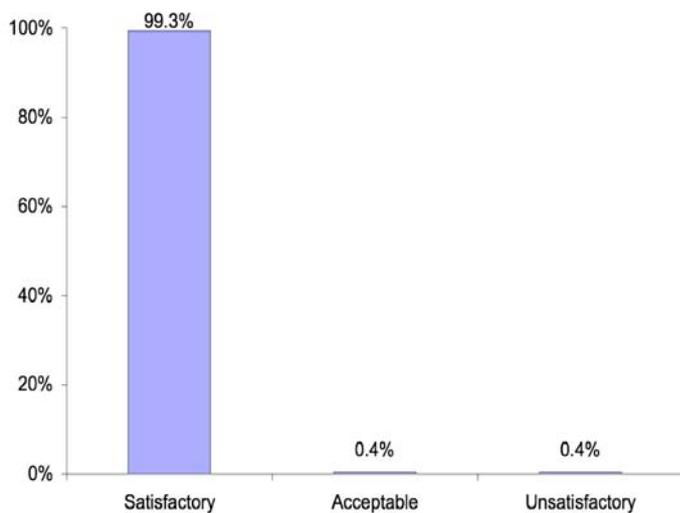


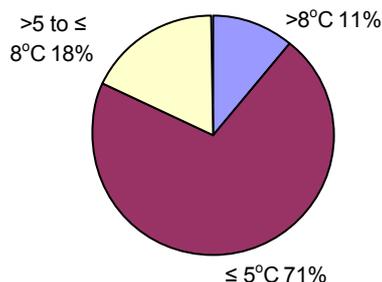
Figure 6: Microbiological classification for coagulase positive staphylococci for samples for which a questionnaire was returned (n=802)



Storage Temperature

The storage/display temperature was provided for 780 samples (Figure 7). Of these, the majority (71%; 554/780) were stored at the recommended temperature of $\leq 5^{\circ}\text{C}$. However, 29% (226/780) of samples were stored at temperatures greater than 5°C (range 5.1°C to 17.9°C). Eleven percent of samples were stored/displayed at temperatures $>8^{\circ}\text{C}$ (range: 8.2°C - 17.9°C , mode= 10.0°C , median= 10.8°C).

Figure 7: Storage/display temperature of sandwiches (n=782)



Four of the five (80%) sandwiches that were categorised as unsatisfactory or unacceptable/potentially hazardous were stored at temperatures $>8^{\circ}\text{C}$ (Table 3). One egg salad sandwich was displayed at ambient temperatures, not in a refrigerated unit (measured temperature 17.9°C) and had an unsatisfactory coagulase positive staphylococci count (310 cfu/g).

Also of concern was the finding that a chicken and stuffing sandwich, which was unacceptable/potentially hazardous for *L. monocytogenes*, was stored at 8.8°C and still had two days remaining until the use by date expired. This could have provided the opportunity for *L. monocytogenes* to multiply to even higher levels.

In the US, a case control study of the potential sources of sporadic illness suggested that retail environments play a role in the contamination of foods and/or amplification of *L. monocytogenes*²⁹. Hunter *et al.* (1990) found that *L. monocytogenes* was isolated from 28% of sandwiches displayed at ambient temperatures, compared to 7% of those stored at lower temperatures, while the counts were very low in chilled sandwiches²⁰.

Inappropriate storage of food in bedside lockers has been reported in investigations into hospital-associated listeriosis outbreaks²¹. The current survey found that 24% (12/50^f) of the sandwiches sampled in health care settings (which could potentially be served to vulnerable people) were stored/displayed at temperatures $>5^{\circ}\text{C}$. Although all were satisfactory for *L. monocytogenes* and coagulase positive staphylococci, there was at least one day remaining until the use-by date⁹ expired, which could have allowed *L. monocytogenes* growth, if present, before consumption.

Similarly, two of the sandwiches which were unsatisfactory for coagulase positive staphylococci were stored at 10°C and still had one day remaining until the end of the use by date. Doubling times of 12 to 24 hours have been reported for *S. aureus* have been reported at 10°C , but this decreases to 1 to 2 hours at 20°C ⁴ so it is important that the chill chain is maintained. Temperature abuse contributed to the UK outbreak of staphylococcal food poisoning in 2009, where sandwiches were stored unrefrigerated in a van on a hot summer day³.

^f 1/17 sandwiches served to patients, 2/5 sandwiches from shop in health care settings, and 9/28 sandwiches sold in the café/restaurant/canteen in healthcare settings were stored/displayed at temperatures $>5^{\circ}\text{C}$

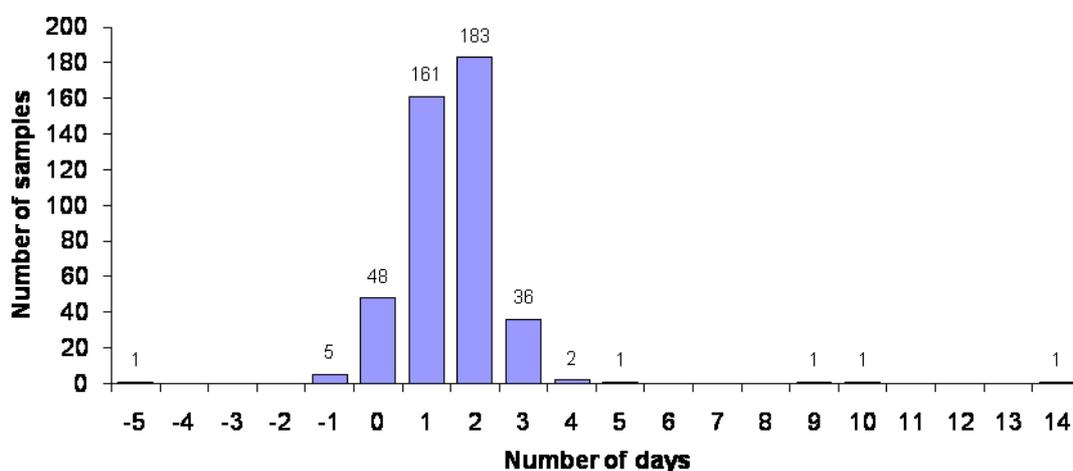
⁹ Information on use-by date only available for sandwiches delivered pre-packaged (7/12)

Use-by Date

Information on where the sandwiches were pre-packaged was provided for 783 samples. Of these, 61% (n=476) were delivered pre-packaged and 39% (n=307) were pre-packaged on site. A use-by date is only required to be labelled on sandwiches which are pre-packaged off-site^h. There was a high level of compliance with this requirement, as a use-by date was labelled on 99% (461/466) of sandwiches that were delivered pre-packaged.

On the questionnaire, the use-by date field was completed for 440 samples (Figure 8). At the time of collection, the majority of sandwiches had one or two days remaining until the use-by date expired. For six sandwiches, the use-by date had expired at the time of collection; by one day (five samples) and by five days (one sample).

Figure 8: For sandwiches delivered pre-packaged: number of days remaining until use by date (n=440)



Three sandwiches had a considerably longer period of time remaining until the use-by date expired (9, 10 and 14 days). The sandwich with nine days remaining was packaged in a modified environment (which can extend the shelf-life of a food product by 7-10 days⁶) but the other two sandwiches were not. From the information provided by the survey questionnaire, it is not possible to determine if these shelf-lives were excessive, but it is the responsibility of the food business operator to produce safe food and to determine an accurate and realistic shelf-life. The British Sandwich Association recommends that the use-by date should be production plus two days, and that where the shelf-life is longer than this, manufacturers must be able to prove that their products are safe, generally by the use of independent shelf-life tests⁷. Both the European Commission and the FSAI have published a guidance documents to assist food business operators determine the shelf life of their products^{12,14}.

Labelling Requirements for Modified Atmosphere Packaging

If a food product's shelf life has been extended by the use of packaging gasses the label must state 'packaged in a protective atmosphere'ⁱ. Information on whether or not the sandwiches were packaged in a modified atmosphere provided for 629 samples. Of these, only 1.3% (n=8) were packaged in a modified atmosphere, indicating that this is not common practice for pre-packaged sandwiches in Ireland.

^h Required under the general labelling legislation (Council Directive 2000/13/EC)

ⁱ Commission Directive 94/54/EEC

Sandwich Fillings

Table 3 provides a description of the fillings for the five sandwiches with unsatisfactory or unacceptable/potentially hazardous results. Deli meats have been identified as vehicles of listeriosis infection during outbreaks and in this survey, both sandwiches which were unsatisfactory for *L. monocytogenes* contained deli meats (one turkey, one chicken). However, due to the range of combinations of sandwich fillings in the 802 sandwiches described by questionnaire, it was not possible to determine a statistical significance for these findings.

Whilst a 1990 survey of pre-packaged sandwiches found no association between the isolation of *Listeria* and sandwich filling²⁰, Little *et al.* (2008) found that the presence of *Listeria* spp. and *L. monocytogenes* was associated with sandwiches that had a main sandwich filling of poultry meat, or contained salad ingredients, soft cheese or mayonnaise²². Little *et al.* (2010) used a source attribution model to quantify the contribution of various foods to human listeriosis in the UK. They found the most important food sources of listeriosis in the overall population and the older population (≥ 60 years old) were multi-component foods (sandwiches and pre-packaged mixed salad vegetables) finfish and beef; for pregnancy-associated cases, beef, milk and milk products, and finfish were the most important sources of infection²⁴.

In the EU member states, the food category with the highest proportion of samples which exceed legal limits for *L. monocytogenes* was ready-to-eat fishery products, followed by ready-to-eat meat products and cheeses¹⁰. Between 2008 and 2009, the FSAI issued three food alerts recalling cooked ham and soft cheese due to the presence of *L. monocytogenes*¹⁶⁻¹⁸. The European Commission intends to coordinate a monitoring programme in order to establish baseline data on the prevalence and level of *L. monocytogenes* contamination in ready-to-eat fishery products, cheeses and heat treated meat products across the EU.

CONCLUSIONS

Only a small proportion of sandwiches tested in this survey were unsatisfactory for coagulase positive staphylococci (0.3%). Although the percentage of sandwiches which were unacceptable/potentially hazardous for *L. monocytogenes* was low (0.2%), the fact that *L. monocytogenes* was detected demonstrates the importance of its control in the manufacturing environment¹⁵. As required by Commission Regulation 2073/2005, environmental sampling for *L. monocytogenes* in plants manufacturing ready-to-eat foods can identify potential contamination sources within the plant to which additional cleaning and disinfection procedures can be targeted.

The survey highlighted issues of temperature control at retail level, selling sandwiches beyond the use-by date at retail level, and the possible application of inappropriate use-by dates by manufacturers. The use of modified atmosphere packaging to extend the shelf-life of sandwiches is not common practice in Ireland.

From the results of the survey, it was not possible to determine an association between sandwich filling and the presence of unsatisfactory levels of coagulase positive staphylococci or unacceptable/potentially hazardous levels of *L. monocytogenes*. However, both sandwiches that were unacceptable/potentially hazardous for *L. monocytogenes* contained poultry meat and mayonnaise, and one contained salad – ingredients which have been associated with the presence of *Listeria* spp. and *L. monocytogenes* in sandwiches²².

The consistent findings of small numbers of *L. monocytogenes* in sandwiches and recent outbreaks associated with the consumption of sandwiches in hospitals reiterate the importance of protecting vulnerable people, particularly elderly people in hospitals or care settings^{2,21}.

RECOMMENDATIONS

From the findings of this study, it is recommended that:

- 1) Retailers ensure that ready-to-eat sandwiches are stored/displayed at $\leq 5^{\circ}\text{C}$
- 2) Retailers ensure that ready-to-eat sandwiches are not sold once their use-by date expires
- 3) Manufacturers ensure that they apply an accurate and realistic shelf-life to ready-to-eat sandwiches and should follow the EU guidance document on *Listeria monocytogenes* shelf-life studies for ready-to-eat foods¹². Where laboratory testing is undertaken to investigate shelf-life, testing should be carried out by an independent and accredited laboratory
- 4) Manufacturers sandwiches continue to control *L. monocytogenes* the in manufacturing environment
- 5) Hospitals and other establishments which cater for the elderly should be aware that pre-packaged sandwiches can be a source of listeriosis and care should be taken to protect vulnerable patients from this risk

REFERENCES

1. UK Advisory Committee on the Microbiological Safety of Food (2009). Report on the increased incidence of listeriosis in the UK. Food Standards Agency. *Ad Hoc* Group on Vulnerable Groups. Available at: <http://www.food.gov.uk/multimedia/pdfs/committee/acmsflisteria.pdf> (accessed 11th May 2010)
2. Anon (2009). Manufacturers and regulators must learn from listeriosis mistakes. eFood (European Food Scientist)17-18. Available at: http://www.scientistlive.com/European-Food-Scientist/Hygiene/Manufacturers_and_regulators_must_learn_from_listeriosis_mistakes/23476/ (accessed 11th May 2010)
3. Anon (2009). Recent staphylococcal foodborne outbreaks. Health Protection Report 3 (29). Available at: <http://www.hpa.org.uk/hpr/archives/2009/news2909.htm#staph> (accessed 11th May 2010)
4. Baird-Parker, T.C. (2010). *Staphylococcus aureus*. In The Microbiological Safety and Quality of Food. Volume I, pages 1317-1335. B.M. Lund, T.C. Baird-Parker, and G. Gould (eds.). Aspen Publishers, Inc.
5. Balaban, N. and Rasooly, A. (2000). Staphylococcal enterotoxins. International Journal of Food Microbiology 61:1-10.
6. BOC (2010). Modified atmosphere packaging. Available at: http://www.boconline.ie/products/products_by_application/food_freezing_and_chilling/modified_atmosphere_pack.asp (accessed 11th May 2010)
7. British Sandwich Association (2007). Manufacturer code of practice. Available at: http://www.sandwichesonline.org.uk/about_the_bsa/manufacturer_code_of_practice.shtml (accessed 11th May 2010)
8. Colombari, V., Mayer, M.D.B., Laicini, Z. M., Mamizuka, E., Franco, B.D.G., Destro, M.T. and Landgraf, M. (2007). Foodborne outbreak caused by *Staphylococcus aureus*: phenotypic and genotypic characterization of strains of food and human sources. Journal of Food Protection 70:489-493.
9. Drevets, D.A. and Bronze, M.S. (2008). *Listeria monocytogenes*: epidemiology, human disease, and mechanisms of brain invasion. FEMS Immunology and Medical Microbiology 53:151-165.
10. EFSA (2009). Report of Task Force on Zoonoses Data Collection on proposed technical specifications for a survey on *Listeria monocytogenes* in selected categories of ready-to-eat food at retail in the EU. The EFSA Journal 2616:1-66.
11. European Commission (2005). COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Available at: http://www.fsai.ie/uploadedFiles/Consol_Reg2073_2005.pdf (accessed 11th May 2010)
12. European Commission (2008). Guidance Document on *Listeria monocytogenes* shelf-life studies for ready-to-eat foods, under Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Available at: http://www.fsai.ie/uploadedFiles/EU_Guidance_listeria_monocytogenes.pdf (accessed 17th June 2010)
13. FSAI (2002). Microbiological safety of pre-packed sandwiches. Available at: http://www.fsai.ie/uploadedFiles/3rdQuarter_prepacked_sandwiches.pdf (accessed 11th May 2010)



14. FSAI (2005). Determination of product shelf-life. Guidance Note 18. Available at: <http://www.fsai.ie/WorkArea/DownloadAsset.aspx?id=756> (accessed 11th May 2010)
15. FSAI (2005). The control and management of *Listeria monocytogenes* contamination of food. Available at: <http://www.fsai.ie/WorkArea/DownloadAsset.aspx?id=1234> (accessed 11th May 2010)
16. FSA (2008). Recall of soft washed rind cheeses from France due to presence of *Listeria monocytogenes*. Available at: <http://www.fsai.ie/details.aspx?id=3116> (accessed 11th May 2010)
17. FSAI (2009). *Listeria monocytogenes* in Rhyme Baked Sliced Ham. Available at: http://www.fsai.ie/news_centre/food_alerts/farneyham.html (accessed at 11th May 2010)
18. FSAI (2009). Recall of SuperValu Homestyle Crumbed Ham and Centra Homestyle Crumbed Ham and Centra Homestyle Cooked Ham. Available at: http://www.fsai.ie/news_centre/food_alerts/fa05092009.html (accessed 11th May 2010)
19. Government of Canada (2009). Report of the Independent Investigator into the 2008 Listeriosis Outbreak. Available at: <http://news.gc.ca/web/article-eng.do?crtr.sj1D=&mthd=advSrch&crtr.mnthndVI=5&nid=468909&crtr.dpt1D=&crtr.tp1D=&crtr.lc1D=&crtr.yrStrtVI=2008&crtr.kw=report%2Bof%2Bthe%2Bindependent%2Binvestigator&crtr.dyStrtVI=26&crtr.aud1D=&crtr.mnthStrtVI=2&crtr.yrndVI=2010&crtr.dyndVI=11> (accessed 11th May 2010)
20. Hunter, P.R., Hornby, H. and Green, I. (1990). The microbiological quality of pre-packed sandwiches. *British Food Journal* 92:15-18.
21. Irvine, N. (2009). The report of the outbreak control team of the investigation of an outbreak of listeriosis in the Belfast Health and Social Care Trust during May to November 2008. Available at: http://www.google.ie/#hl=en&source=hp&q=The+report+of+the+outbreak+control+team+of+the+investigation+of+an+outbreak+of+listeriosis+in+the+Belfast+Health+and+Social+Care+Trust+during+May+to+November+2008&aq=f&aql=&aql=&oq=&gs_rfai=&fp=cc603a2999465f6e (accessed 11th May 2010)
22. Little, C.L., Barrett, N.J, Grant, K. and McLauchlin, J. (2008). Microbiological safety of sandwiches from hospitals and other health care establishments in the United Kingdom with a focus on *Listeria monocytogenes* and other *Listeria* species. *Journal of Food Protection* 71:309-318.
23. Little, C.L., Mitchell, R.T., and Barnes, J. (2002). Microbiological examination of food from take-aways and sandwich bars. Food Standards Agency Catering Hygiene Initiative. Available at: <http://www.food.gov.uk/multimedia/pdfs/microexamfood.pdf> (accessed 11th May 2010)
24. Little, C.L., Pires, S., Gillespie, I.A., Grant, K. and Nichols, G.L. (2010). Attribution of human *Listeria monocytogenes* infections in England and Wales to ready-to-eat food sources placed on the market: adaptation of the Hald *Salmonella* source attribution model. *Foodborne Pathogens and Disease* 7:1-8.
25. Little, C.L., Sagoo, S.K., Gillespie, I.A., Grant, K. and McLauchlin, J. (2009). Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in selected retail ready-to-eat foods in the United Kingdom. *Journal of Food Protection* 72:1869-1877.
26. Meldrum, R.J., Mannion, P.T. and Garside, J. (2009). Microbiological quality of ready-to-eat food served in schools in Wales, United Kingdom. *Journal of Food Protection* 72:197-201.
27. Meldrum, R.J. and Smith, R.M.M. (2007). Occurrence of *Listeria monocytogenes* in sandwiches available to hospital patients in Wales, United Kingdom. *Journal of Food Protection* 70:1958-1960.



28. Todd C.D., Greig J.D., Bartleson C.A., and Michaels B.S. (2008). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 4. Infective doses and pathogen carriage. *Journal of Food Protection* 71:2339-2373.
29. Varma, J.K., Samuel, M.C., Marcus, R., Hoekstra, R.M., Medus, C., Segler, S., Anderson, B.J., Jones, T.F., Shiferaw, B., Haubert, N., Megginson, M., McCarthy, P., Graves, V.L., Gilder, T. and Angulo, F.J. (2007). *Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. *Clinical Infectious Diseases* 44:521-528.
30. Warriner, K. and Namvar, A. (2009). What is the hysteria with *Listeria*? *Trends in Food Science & Technology* 20:245-254.



APPENDIX 1: QUESTIONNAIRE

APPENDIX 2: Questionnaire 09NS1
Microbiological safety of pre-packaged sandwiches

Please note: 1) EHOs must complete this questionnaire for all samples, 2) all questions are mandatory & 3) all questionnaires must be returned to the FSAI by 11/09/2009

1. General Information:

* EHO Name: _____ * EHO Sample Reference Number (i.e. EHO's own personal reference number for the sample) _____

* Laboratory Reference Number (upon receipt of lab report) _____

2. Sample source (See section 3 of Protocol):

Retail: Supermarket , Corner shop , Farmers market , Vending machine , Forecourt , Shop within healthcare setting , Other (please specify: _____)

Service sector: Restaurant , Hotel , Café , Take-away , Café/restaurant/canteen within health care setting , Sandwiches served to patients in Hospital , Sandwiches served to patients in Nursing Home , Other (please specify: _____)

3. Storage conditions (See section 6.5 of Protocol):

Storage conditions: Refrigerated or Other (please specify: _____), **Storage Temperature, i.e. temperature (measured by EHO) of storage/display unit:** _____ °C

4. Sample information (See section 4 of Protocol):

a) Was the sandwich 'delivered pre-packaged' (state the manufacturer/brand name: _____) **or** was the sandwich 'pre-packaged on site'

b) For sandwiches 'delivered pre-packaged' did the label provide a use-by date: Yes (please specify the date: _____) **or** No

c) Did the label state that the sandwich was packaged in a modified environment: Yes **or** No

5. Sandwich fillings (please tick as many boxes as necessary):

Meat:	Poultry:	Seafood:	Cheese:	Cheese:	Other:
Beef <input type="checkbox"/>	Chicken <input type="checkbox"/>	Smoked salmon <input type="checkbox"/>	Hard cheese <input type="checkbox"/>	Pasteurised <input type="checkbox"/>	Egg <input type="checkbox"/>
Ham <input type="checkbox"/>	Turkey <input type="checkbox"/>	Tuna <input type="checkbox"/>	Soft cheese <input type="checkbox"/>	Not pasteurised <input type="checkbox"/>	Coleslaw <input type="checkbox"/>
Pork <input type="checkbox"/>	Other <input type="checkbox"/>	Other <input type="checkbox"/>	Unknown <input type="checkbox"/>	Unknown <input type="checkbox"/>	Salad <input type="checkbox"/>
Other <input type="checkbox"/>					Mayonnaise <input type="checkbox"/>
					Other <input type="checkbox"/>

6. Follow-up action (section 9 of Protocol):
 (Please tick as many boxes as necessary)

None

Product recall

Repeat sample Lab. ref. no.: _____

Other action (please specify): _____



APPENDIX 2: SAMPLES SUBMITTED BY HSE AREA

HSE Region	HSE area	Samples Submitted
Dublin/ Mid-Leinster	East Coast Area	41
	Midlands Area	91
	South Western Area	86 ^a
Dublin/North Eastern	North Eastern Area	95 ^b
	Northern Area	78
Western Region	Mid-Western Area	105
	North Western Area	72 ^c
	Western Area	98
Southern Region	South Eastern Area	179
	Southern Area	126
Total		971

23 samples (4^a; 7^b and 12^c) were excluded from analysis as they did not meet the survey protocol regarding sample month



APPENDIX 3: RANGE OF MICROBIOLOGICAL COUNTS OF SAMPLES

<i>L. monocytogenes</i> count (cfu/g)	Classification	Number of samples	% of samples
<10	Satisfactory	276	29.1%
<20	Satisfactory	664	70.0%
30	Satisfactory	1	0.1%
40	Satisfactory	1	0.1%
50	Satisfactory	1	0.1%
90	Satisfactory	2	0.2%
<100	Satisfactory	1	0.1%
430	Unacceptable/potentially hazardous	1	0.1%
2,900	Unacceptable/potentially hazardous	1	0.1%
Total		948	100

<i>Coagulase positive staphylococci</i> count (cfu/g)	Classification	Number of samples	% of samples
<10	Satisfactory	333	35.1%
10	Satisfactory	3	0.3%
<20	Satisfactory	606	63.9%
30	Acceptable	1	0.1%
54	Acceptable	1	0.1%
90	Acceptable	1	0.1%
120	Unsatisfactory	1	0.1%
150	Unsatisfactory	1	0.1%
310	Unsatisfactory	1	0.1%
Total		948	100



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