

3rd Quarter National Microbiology Survey 2002 (NS3):

Microbiological safety of pre-packed sandwiches

TABLE OF CONTENTS

	Summary	3
1.	Introduction	3
2.	Specific Objectives	5
3.	Method	5
	3.1 Sample description	5
	3.2 Sample collection	5
	3.3 Sample analysis	6
4.	Results and Discussion	7
	4.1 Microbiological safety	7
	4.1.1 <i>Staphylococcus aureus</i>	7
	4.1.2 <i>Listeria monocytogenes</i>	9
	4.2 Sample information	12
	4.2.1 Location of preparation: 'on-site' or 'delivered'?	12
	4.2.2 Temperature	13
5.	Conclusions	15
6.	Bibliography	16
7.	Appendices	18

Summary

This study investigated the microbiological safety of 475 pre-packed sandwiches obtained from retail premises which had been prepared and packed on-site or which had been delivered from off-site manufacturers.

Samples were analysed for *Staphylococcus aureus* and *Listeria monocytogenes*. Applying the national microbiological guidelines for ready-to-eat foods to the results of this survey show that :

- 97.7% (464/475) of samples were satisfactory, 1.7% (8/475) acceptable and 0.6% (3/475) unsatisfactory for *S. aureus*
- 98.8% (319/323) of samples were satisfactory, 0.9% (3/323) acceptable and 0.3% (1/323) unacceptable/potentially hazardous for *L. monocytogenes*
- the location of manufacture (i.e. on or off site) had no impact on the microbiological safety of the samples.

Of concern was the finding that the core temperature of 55.9% (94/168) control samples was $>5^{\circ}\text{C}$. These temperatures are not in keeping with current recommendations that ready-to-eat foods should be maintained at a temperature $\leq 5^{\circ}\text{C}$.

1. Introduction

The sale of pre-packed sandwiches from retail premises has increased dramatically over the past few years.

The popularity of sandwiches may be attributed to a number of factors including the evolution of the 'convenience generation'. People of this generation lead hectic lifestyles and work long hours; more women work outside the home and more people live alone. These factors have impacted on eating habits and have lead to 1) an increase in the demand for convenience foods which can be 'eaten on the go' or which can be readily assembled and 2) an increase in the number of meals eaten outside of the home.

Consumer demand has lead to an increase in the availability of sandwiches made from speciality breads, complex fillings and complex combination of fillings. Therefore although the sandwich is a simple product, it is complex in terms of the interactions and microbiology of the ingredients ⁽¹⁾. This complexity means that

quality, safety and hygiene issues must be taken into account before, during and after production ⁽²⁾.

In terms of food safety, food poisoning outbreaks have been associated with the consumption of sandwiches. Outbreaks caused by *Salmonella* spp. ⁽³⁾, Norwalk like viruses ⁽⁴⁾, Verocytotoxigenic *E. coli* (VTEC) ⁽⁵⁾ and *Staphylococcus aureus* ⁽⁶⁾ are among those reported.

In this study the bacteriological safety (*S. aureus* and *Listeria monocytogenes*) of pre-packed sandwiches available at retail level was assessed. *S. aureus* is a bacterium which is commonly associated with the skin, nose and throat of healthy individuals. Its presence in food is indicative of poor hygiene and handling practices. Staphylococcal food poisoning is caused by ingestion of a toxin formed by *S. aureus* in the food. *S. aureus* must grow to levels of $>10^5$ cells/g before producing sufficient quantities of the heat-stable staphylococcal toxin to cause illness. The onset of symptoms is usually rapid (1 to 7 hours after ingestion of the food containing the toxin), however, both the onset and the severity of the symptoms depend on the persons susceptibility and the amount of toxin they have consumed. The main symptoms include abdominal cramps, vomiting and diarrhoea. Generally no treatment is required except in severe cases where anti-dehydration treatments may be required.

L. monocytogenes is a bacterium which is ubiquitous in the environment and is present in many raw foods. A number of foods have been implicated as vehicles in the transmission of human listeriosis[¥] including coleslaw, cheese, pate, deli meats and smoked fish. These foods are commonly used as sandwich fillings. Of additional concern is the ability of this bacterium to grow at refrigeration temperatures (i.e $<5^{\circ}\text{C}$). Concern is raised when the level of this bacterium exceeds 100cfu/g at the point of consumption ⁽⁷⁾. Symptoms range from a mild flu-like condition to severe life-threatening infections characterised by septicaemia and meningoencephalitis. Pregnant women, neonates, the elderly and immunocompromised are particularly vulnerable. Infection during pregnancy can result in abortion or stillbirth.

[¥] Listeriosis is the disease caused by *L. monocytogenes*.

2. Specific objectives

The aim of this study was to examine the microbiological safety of pre-packed sandwiches with respect to *S. aureus* and *L. monocytogenes*.

3. Method

3.1 Sample description

Two categories of sandwich were included in this study: 1) pre-packed sandwiches prepared and packed on-site, and 2) 'delivered' sandwiches prepared and packed off-site. All samples were stored and displayed under refrigerated conditions. Sampling focused on sandwiches with fillings that were likely to have received considerable post cooking handling (eg. cooked meats) and/or have been associated with *L. monocytogenes* contamination (eg. soft cheese, deli meats, coleslaw, and seafood). Effort was made to select sandwiches closest to their 'use by date' and those prepared immediately prior to sale were excluded from this study.

3.2 Sample collection

Environmental Health Officers (EHOs) from the 10 Health Boards collected a total of 475 samples from retail premises selling pre-packed sandwiches (Appendix 1). Most samples were collected in July and August 2002, however 69 of the 475 samples were collected during September 2002. In general, only one sample was collected per premises, but in premises where sandwiches from different manufacturers were sold, collection of a second sample was permitted. The minimum sample size was 75 g.

At the time of sample collection EHOs were requested to complete the following information on the sample submission form or on the accompanying questionnaire:

- The location of manufacture, i.e pre-packed 'on-site' or 'delivered',
- The name of the manufacturer (for 'delivered' sandwiches),
- The use by date,
- A description of the sandwich filling, and
- The internal temperature (i.e. the core temperature) of a control sandwich. To ensure that this temperature was representative of the test sandwich, a control sandwich was selected which was displayed beside the test sandwich, had the same filling, batch number and/or use by date.

3.3 Sample analysis

Samples were submitted to one of the seven Official Food Microbiology Laboratories (Appendix 2), and analysed using approved/standard methods for the following parameters:

- *S. aureus* (enumeration)
- *L. monocytogenes* (detection and enumeration)

The microbiological safety of the samples was determined using the Irish microbiological guidelines for ready-to-eat foods ⁽⁸⁾ (Table 1).

Table 1: Microbiological safety based on the Irish microbiological guidelines for ready-to-eat foods ⁽⁸⁾

Parameter	Microbiological safety (cfu/g)			
	Satisfactory	Acceptable	Unsatisfactory	Unacceptable potentially hazardous
<i>L. monocytogenes</i>	<20	20 - <100	N/A	≥100
<i>S. aureus</i>	<20	20 - <100	100 - <10 ⁴	≥10 ⁴

N/A = not applicable

4. Results and Discussion

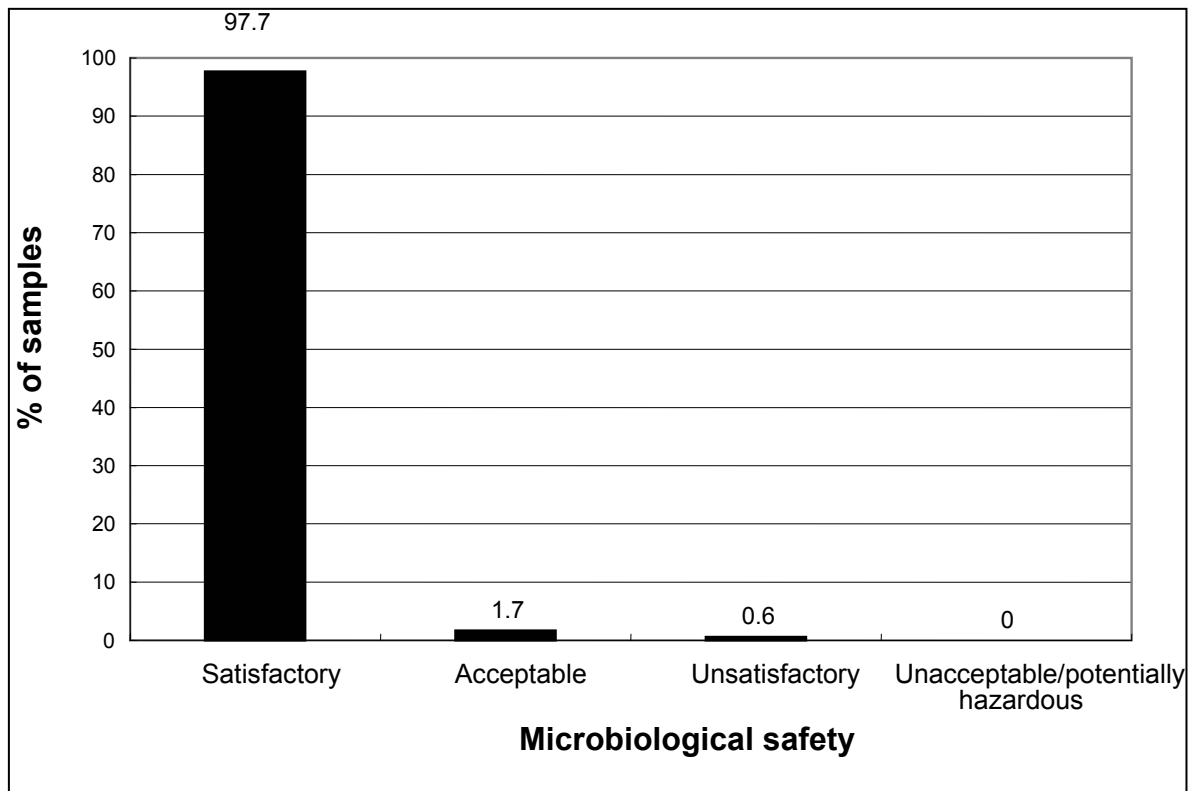
4.1 Microbiological safety

A total of 475 samples from the 10 health boards were analysed for 1 or more microbiological parameter (*S. aureus* and *L. monocytogenes*). Details of the numbers submitted from each health board are presented in Appendix 1.

4.1.1 *Staphylococcus aureus*

Of the 475 samples assessed quantitatively for *S. aureus*, 97.7% (n=464) were satisfactory, 1.7% (n=8) were acceptable and 0.6% (n=3) were unsatisfactory. No sample was categorised as unacceptable/potentially hazardous (Figure 1). More detailed results by health board area are presented in Appendix 3.

Figure 1: Microbiological safety (*S. aureus*) of samples



Sandwich preparation involves considerable handling during preparation of the filling and sandwich assembly. This handling may increase the risk of

contamination with *S. aureus* unless good hygiene practices are undertaken. This has been demonstrated in an Italian study where *S. aureus* was detected in finished sandwiches prepared from ingredients which were not previously contaminated ⁽⁹⁾.

It is worth noting that 7 of the 11 samples (63.6%) which were categorised as acceptable or unsatisfactory contained salad as a filling, however the statistical significance of this finding could not be determined (due to the combinations and complexity of fillings used in the manufacture of the 475 sandwiches analysed). Salad receives considerable handling during preparation and therefore has the potential to carry high numbers of *S. aureus*. The inclusion of salad as a sandwich filling has also been associated with an increase in the bacterial count (standard plate count) ⁽¹⁰⁾ and an increase in the incidence of *Listeria* spp. (although this was not found to be statistically significant) ⁽¹¹⁾.

The incidence of *S. aureus* at unsatisfactory levels (0.6% >100cfu/g) is similar (p<0.05) to that of a UK study which was undertaken as part of the 1993 EC co-ordinated food control programme ⁽¹²⁾ (Table 2). In a more recent UK survey ⁽¹³⁾ and in an Australian survey ⁽¹⁰⁾ *S. aureus* was detected at unsatisfactory levels (>100cfu/g) in 2% and 11% of samples respectively.

Table 2: Comparison with other studies – Incidence of *S. aureus* at levels >100cfu/g

Origin	Sampling period	Sample description	No. of samples enumerated	No. of samples >100 cfu/g
Australia ⁽¹⁰⁾	1998	Sandwiches and rolls	62	7 (11%)
UK ⁽¹⁴⁾	1989	Sandwiches with various fillings	91	0 (0%)
UK ⁽¹²⁾	1993	Sandwiches with different fillings	324	2 (0.5%)
UK ⁽¹³⁾	2001	Chicken sandwiches and rolls (without salad)	449	10 (2%)
IRL (this study)	2003	Sandwiches with various fillings	475	3 (0.6%)

4.1.2 *Listeria monocytogenes*

L. monocytogenes was assessed qualitatively in 475 samples and was detected in 11% (n=52) of samples. Detailed results by health board are outlined in Appendix 4.

Quantitative tests for *L. monocytogenes* were carried out on 323 of the 475 samples. Of the 323 samples enumerated, 319 samples (98.8%) were satisfactory, 3 samples (0.9%) were acceptable and 1 sample (0.3%) was unacceptable/potentially hazardous (Figure 2). *L. monocytogenes* was detected at a level of 1.2×10^3 cfu/g in the unacceptable/potentially hazardous sample. Results by health board are presented in Appendix 5.

Figure 2: Microbiological safety (*L. monocytogenes*) of samples

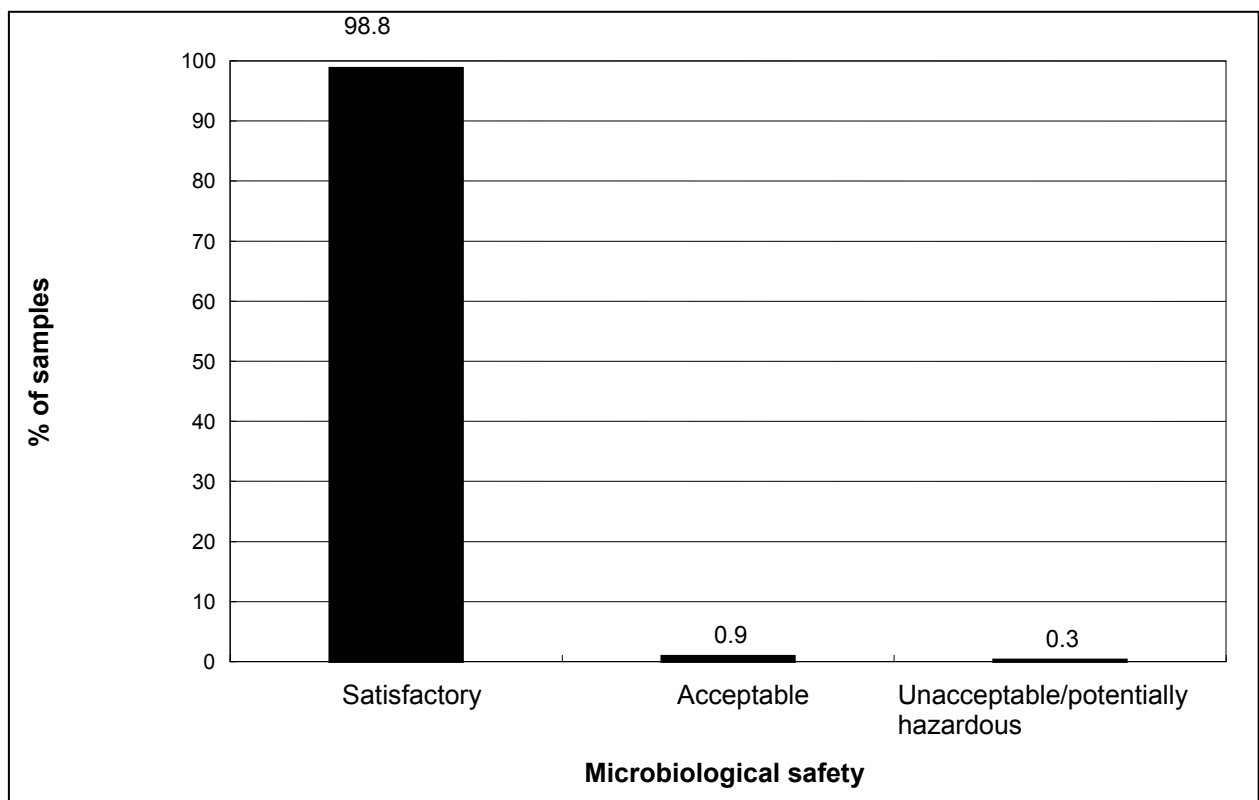


Table 3 details the findings of a number of other surveys which have undertaken qualitative and/or quantitative studies for *L. monocytogenes*.

Table 3: Comparison with other studies – Quantitative and qualitative analysis for *L. monocytogenes*

Origin	Year of study	Sample description	Qualitative tests		Quantitative test	
			No. analysed	<i>L. monocytogenes</i>	No. enumerated	Count
Australia ⁽¹⁰⁾	1998	Sandwiches and rolls	44	1 (2.3)	ND	NA
UK ⁽¹²⁾	1993	Sandwiches with different fillings	324	6 (2%)	ND	NA
UK ⁽¹⁴⁾	1989	Prepacked sandwiches	91	16 (17%)	16	<200 (n=7; 43.75%) ≥200 (n=9; 56.25%)
NI ⁽¹¹⁾	1996 ^Y	Prepacked sandwiches	725	113 (15.59) [♦]	725	≥100 (n=5; 0.68%)
IRL (This study)	2003	Prepacked sandwiches	475	52 (10.9%)	323	≥100 (n=1; 0.3%)

ND: Not Determined

NA: Not Applicable

^Y Year of publication of report

[♦] The incidence of *Listeria* spp. rather than *L. monocytogenes* was recorded in this study

The qualitative results presented in Table 3 show the variability in the incidence of *L. monocytogenes* between studies (ranging from 2 to 17%). This may be related to the nature of the sandwich filling. In a FDA/USDA draft risk assessment on *L. monocytogenes* among selected categories of ready-to-eat foods ⁽¹⁵⁾, seafood, fresh produce, dairy and deli products have been identified as high risk. These foods are commonly used in the preparation of sandwich fillings, however their use as fillings in the studies outlined in Table 3 is unknown (this information was not always reported). In this study, the 4 samples which were categorised as acceptable or unacceptable/potentially hazardous contained ham as a filling. The statistical significance of this finding could not be determined due to the combinations and complexity of fillings used in the manufacture of the 475 sandwiches analysed.

L. monocytogenes is a bacterium which is ubiquitous in the environment and is present in many raw foods, however, its presence in heat processed ready-to-eat foods is usually as a direct result of post process contamination from the processing environment ⁽¹⁶⁾. Processing environments often harbour their own unique population of *L. monocytogenes*⁽¹⁷⁾ which are capable of persisting in the environment over time ^(17, 18). This may be explained by the ability of *L. monocytogenes* to form biofilms⁽¹⁸⁾, survive in niches and adhere to surfaces⁽¹⁹⁾. The potential for post process contamination in the processing plant of heat processed ready-to-eat foods highlights the necessity for 1) processors to implement a *L. monocytogenes* monitoring and control programme and 2) sandwich manufacturers to deal with reputable suppliers of processed ready-to-eat foods. An audit of suppliers should be carried out on a regular basis to ensure that product specifications are being met and that the safety and quality of the processed ready-to-eat food has not been compromised.

4.2 Sample Information

Sample information was captured by means of a questionnaire which was completed at the time of sampling. Questionnaires were returned for 176 of the 475 survey samples, i.e. there was a 37% response rate.

4.2.1 Location of preparation: 'on-site' or 'delivered'?

Of the 176 samples returned with a completed questionnaire, 47% (n=83) were prepared on-site and 53% (n=93) were delivered. The microbiological safety of the samples based on the location of preparation is presented in Table 4.

Table 4: Effect of preparation location on microbiological safety

Location of preparation	No. of samples	<i>S. aureus</i>			<i>L. monocytogenes</i>		
		S*	A*	U*	S*	A*	U/PH*
On site	83	82	1	0	82	0	1
Delivered	93	92	0	1	92	1	0
Total	176	174	1	1	174	1	1

* S=Satisfactory; A=Acceptable; U=Unsatisfactory; U/PH=Unacceptable/potentially hazardous

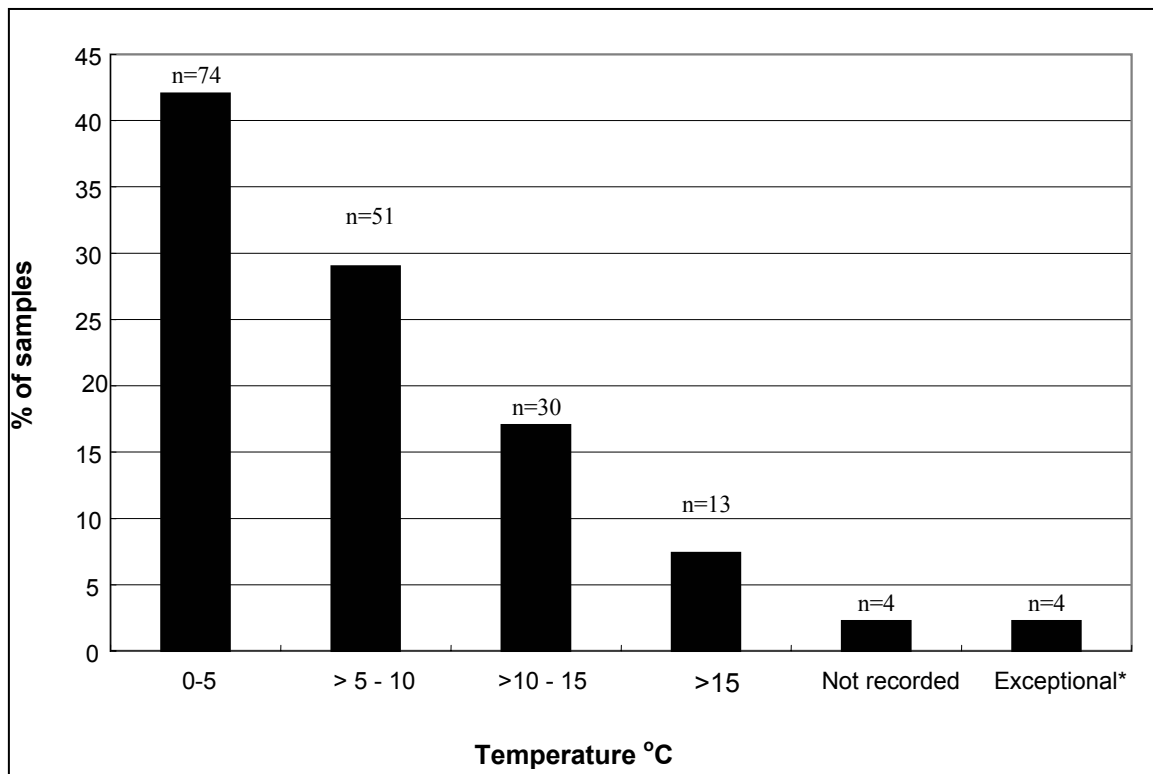
These findings show no statistical difference ($p < 0.05$) between the microbiological safety of sandwiches which were prepared on-site and sandwiches which were delivered (i.e. between sandwiches prepared in retail and manufacturing premises). The categorisation of the majority of samples as satisfactory suggests that food safety controls are in place in most premises; however, more emphasis must be placed on food safety controls in premises where samples with a quality other than satisfactory were obtained.

In a UK study on the microbiological quality of sandwiches from sandwich bars⁽¹³⁾ acceptable microbiological quality was associated with premises where a documented hazard analysis system was in place. In addition, where the manager had received some form of food hygiene training it was more likely that this system was in place. In Ireland it has been a legal requirement since 1998 for all food businesses to implement a food safety management system based on the principles of HACCP⁽²⁰⁾. An assessment on compliance with HACCP in the premises where sampling took place was not undertaken as part of this study.

4.2.2 Temperature

The core temperature of a control sample was recorded for 172 out of the 176 samples which were returned with a questionnaire (Figure 3). This temperature was taken at the time of sampling.

Figure 3: Temperature of control sample at the time of sampling (n= 176)



*Exceptional: samples which cannot be otherwise classified, e.g. '15°C (air temp)', 'Ambient', 'Room Temp', and '>8.4°C'

In terms of food safety, temperature control is essential during the storage and transportation of ready-to-eat foods to minimise the growth of pathogens. It is a crucial control step in any food safety management system. Current recommendations are that ready-to-eat foods should be maintained at a temperature $\leq 5^{\circ}\text{C}$ ⁽²¹⁾. This temperature inhibits the growth of mesophilic[∞] pathogens (e.g. *S. aureus*) and retards the growth of psychrotrophic* pathogens (e.g. *L. monocytogenes*). Despite these recommendations the core temperature

[∞] Mesophiles grow well at ordinary temperatures.

* Psychrotrophs grow well at refrigeration temperatures.

of 55.9% (94/168) control samples was >5°C. Of great concern is the finding that 7.7% (13/168) of control samples had a core temperature >15°C.

The effect of control temperature on the microbiological safety of the samples is presented in Table 5.

Table 5: Effect of control temperature on microbiological safety of the samples

Temperature of control	No. of controls	<i>S. aureus</i>			<i>L. monocytogenes</i>		
		S	A	U	S	A	U/PH
0-5	74	73	0	1 [†]	74	0	0
>5-10	51	51	0	0	51	0	0
>10-15	30	30	0	0	30	0	0
>15	13	13	0	0	11	1 [*]	1 [‡]
Total	168	167 (99.4%)	0	1 (0.6%)	166 (98.8%)	1 (0.6%)	1 (0.6%)

S=Satisfactory; A=Acceptable; U=Unsatisfactory; U/PH=Unacceptable/potentially hazardous

[†]Temperature recorded =5°C

^{*} Temperature recorded = 15.9 °C

[‡] Temperature recorded = 15.4 °C

Considering that the core temperature of 55.9% (94/168) of controls was >5°C, it may appear surprising that 99.4% and 98.8% of samples were satisfactory for *S. aureus* and *L.monocytogenes* respectively. However, microbiological safety is affected not only by temperature but also by the time the food has been exposed to that temperature and the initial level of contamination (i.e. the level of contamination following processing/manufacturing). This information was not collated in this survey.

While no statistical inference was made regarding the data presented in Table 1 it is worth noting that temperatures of 15.9 and 15.4°C were recorded for the controls of the samples which were acceptable and unacceptable/potentially hazardous for *L. monocytogenes*. In addition, the acceptable sample was analysed 2 days before its use by date suggesting that if storage continued at this temperature, *L. monocytogenes* may have proliferated to

unacceptable/potentially hazardous levels by the end of the shelf life. The use-by date was not recorded for the unacceptable/potentially hazardous sample.

5. Conclusions

Food manufacturers/processors, distributors and retailers play an integral role in ensuring the bacteriological safety of food available to the consumer. They also have a legal responsibility in this regard.

The findings of this survey suggest that although good hygiene and handling practices are undertaken in most premises where sandwiches were manufactured; more emphasis is required in premises from which samples were found to be unsatisfactory for *S. aureus*. The presence of *L. monocytogenes* in 0.9% (3/323) of samples at acceptable levels and in 0.3% (1/323) of samples at unacceptable/potentially hazardous levels highlights the need for more emphasis to be placed on the control of this pathogen. It is essential for a *L. monocytogenes* monitoring and control programme to be implemented in both processing and retail environments.

The bacteriological safety of a product at retail level is related to both the initial level of contamination following processing/manufacturing and the additional food safety controls which are undertaken during distribution and storage. Emphasis must be placed on temperature control. Temperature control is required to ensure that the microbiological safety does not deteriorate during distribution and storage. Refrigeration temperatures inhibit the growth of *S. aureus* and thus reduce the risk of toxin production (ingestion of this toxin causes illness). Although *L. monocytogenes* is a psychrotroph, refrigeration temperatures retard its growth and minimise the risk of this pathogen (if present) proliferating to levels >100cfu/g.

In this study, it was difficult to establish the significance of fillings on the microbiological quality of the samples due to the complexity of the combination of fillings used. In future studies it may be more beneficial to concentrate on sandwiches prepared with a smaller more defined range of fillings.

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7. Appendices

Appendix 1: Number of survey samples analysed for 1 or more microbiological parameter from each Health Board (n = 475)

Health Board	Abbreviation	Number of samples
East-Coast Area Health Board	ECAHB	22
Midland Health Board	MHB	25
Mid-Western Health Board	MWHB	46
Northern Area Health Board	NAHB	59
North-Eastern Health Board	NEHB	31
North-Western Health Board	NWHB	38
South-Eastern Health Board	SEHB	89
Southern Health Board	SHB	82
South-Western Area Health Board	SWAHB	51
Western Health Board	WHB	32
Total		475

Appendix 2: List of the Official Food Microbiology Laboratories (OFMLs)

Laboratory

Cherry Orchard Hospital
Mid-Western Regional Hospital
Public Analysts Laboratory, Dublin
Sligo General Hospital
St. Finbarr's Hospital, Cork
University College Hospital, Galway
Waterford Regional Hospital

Appendix 3: *S. aureus* results by health board (n = 475)

Health board	Number of Survey samples	Microbiological quality (cfu/g)			
		Satisfactory (%)	Acceptable (%)	Unsatisfactory (%)	Unacceptable potentially hazardous (%)
		< 20	20 - < 100	100 - < 10 ⁴	≥ 10 ⁴
ECAHB	22	22 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
MHB	25	23 (92.0)	2 (8.0)	0 (0.0)	0 (0.0)
MWHB	46	45 (97.8)	1 (2.2)	0 (0.0)	0 (0.0)
NAHB	59	58 (98.3)	1 (1.7)	0 (0.0)	0 (0.0)
NEHB	31	31 (100)	0 (0.0)	0 (0.0)	0 (0.0)
NWHB	38	36 (94.7)	0 (0.0)	2 (5.3)	0 (0.0)
SEHB	89	88 (98.9)	1 (1.1)	0 (0.0)	0 (0.0)
SHB	82	80 (97.6)	1 (1.2)	1 (1.2)	0 (0.0)
SWAHB	51	49 (96.0)	2 (4.0)	0 (0.0)	0 (0.0)
WHB	32	32 (100)	0 (0.0)	0 (0.0)	0 (0.0)
Total	475	464 (97.7)	8 (1.7)	3 (0.6)	0 (0.0)

Appendix 4. *Listeria* qualitative results by health board (n = 475)

Health board	Survey samples	Number of Qualitative tests	<i>L. monocytogenes</i> (%)
ECAHB	22	22	3 (13.6)
MHB	25	25	12 (48.0)
MWHB	46	46	3 (6.6)
NAHB	59	59	6 (10.2)
NEHB	31	31	1(3.2) ^a
NWHB	38	38	1 (2.6)
EHB	89	89	19 (21.3)
SHB	82	82	5 (6.1)
SWAHB	51	51	2 (3.9)
WHB	32	32	0 (0.0)
Total	475	475	52 (11)

^aBoth *L. monocytogenes* and *L. innocua* were detected in this sample

Appendix 5: *L. monocytogenes* quantitative results by health board (n = 325)

Health board	Survey samples	Quantitative tests	Satisfactory (%) < 20	Acceptable (%) 20 - < 100	Unacceptable/potentially hazardous (%) ≥ 100
ECAHB	22	9 [¥]	9 (100.0)	0 (0.0)	0 (0.0)
MHB	25	25	23 (92.0)	1 (4.0)	1 (4.0)
MWHB	46	3 [™]	3 (100.0)	0 (0.0)	0 (0.0)
NAHB	59	27 [¥]	27 (100.0)	0 (0.0)	0 (0.0)
NEHB	31	0 [¥]	-	-	-
NWHB	38	38	38 (100)	0 (0.0)	0 (0.0)
SEHB	89	87 [§]	86 (98.8)	1 (1.2)	0 (0.0)
SHB	82	82	81 (98.8)	1 (1.2)	0 (0.0)
SWAHB	51	20 [¥]	20 (100.0)	0 (0.0)	0 (0.0)
WHB	32	32	32 (100)	0 (0.0)	0 (0.0)
Total	475	323	319 (98.8)	3 (0.9)	1 (0.3)

[¥] Samples from these areas were submitted to Cherry Orchard Hospital and the Public Analysts Laboratory (Dublin). Samples submitted to Cherry Orchard Hospital were not enumerated

[™] Enumeration was only carried out on the 3 samples in which *L. monocytogenes* was detected qualitatively

[§] Enumeration results were not available for 2 samples