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Occurrence of *Listeria* species in prepacked retail sandwiches

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SUMMARY

A survey of 725 prepacked sandwiches was conducted examining for the presence on enrichment, and by plate count, of *Listeria* species. Sandwiches were found to contain *Listeria* sp. more frequently than their component foods. Chicken, beef and bacon fillings were associated with more frequent isolation. Salad also was associated with more frequent isolation, but the increase was not significant. On enrichment, over 15% of sandwiches contained *Listeria* species. *L. innocua* and *L. monocytogenes* were the only species isolated by plate count at numbers $\geqslant 100$ cfu/g (1·5% of total samples). Potentially hazardous levels of *L. monocytogenes* (defined as $\geqslant 10^3$ cfu/g) were found in two sandwiches examined (0·3%), indicating that although Total Viable Counts (TVCs) may often be high, the risk of listeriosis to vulnerable individuals from sandwiches is relatively low. It is important to distinguish the risk of consuming potentially hazardous levels of a pathogen in food from the risk of contracting illness as a result of such an event.

INTRODUCTION

Listeria monocytogenes is capable of causing serious and life-threatening illnesses including septic abortion, meningoencephalitis and septicaemia in immuno-compromised individuals [1]. Outbreaks have been traced to foods such as soft cheese and pâté [2, 3], but many cases are sporadic and cannot be linked to any specific food [1].

Prepacked sandwiches are an increasingly popular food which may contain a wide range of single and mixed fillings. Previous surveys have shown such sandwiches frequently have high TVCs and, less frequently, a range of potential pathogens including pathogenic *Listeria* spp. [4–6]. Such surveys have highlighted a lack of awareness among retail staff concerning the importance of temperature control in the display of sandwiches [4].

Difficulty often exists in conducting an effective analysis following microbiological examination because of the complexity of the various combinations of fillings which may be used in the manufacture of these products [5]. For surveys to provide useful microbiological data on these potentially complex food environments they must concentrate either on a small and defined range of fillings, or examine very large numbers of samples.

This retrospective survey was undertaken to assess if prepacked sandwiches present a significant risk of exposure to *Listeria* spp. in vulnerable individuals.

MATERIALS AND METHODS

Prepacked sandwiches, manufactured by a number of different producers, were purchased from retail display in a variety of premises including supermarkets, petrol stations, cafés and small shops, and transported to the laboratory in cool boxes at < 5 °C.

Bread and filling were aseptically weighed out and examined together by enriching for *Listeria* sp. using the following method [7]. For all foods, 25 g was weighed into 225 ml Listeria Enrichment Broth

Table 1. *Detection of* Listeria *species in sandwiches by enrichment*

	Sandwich			Filling†				
Food	*n*	*N*	Percent	*n*	*N*	Percent	*Z*-value	Significance
Bacon	2	4	50·00	0	20	0·00	3·30	***
Bacon + salad	3	19	15·79	—	—	—	—	—
Beef	4	19	21·05	44	1295	3·40	4·07	***
Beef + salad	6	29	20·69	—	—	—	—	—
Cheese	0	6	0·00	2	49	4·08	−0·50	NS
Cheese + salad	1	10	10·00	—	—	—	—	—
Chicken	25	137	18·25	105	949	11·06	2·42	*
Chicken + salad	17	89	19·10	—	—	—	—	—
Egg	7	31	22·58	2	34	5·88	1·95	NS
Egg + salad	15	69	21·78	—	—	—	—	—
Seafood	1	25	4·00	7	49	14·29	−1·35	NS
Seafood + salad	6	44	13·64	—	—	—	—	—
Ham	3	56	5·36	74	1141	6·49	−0·34	NS
Ham + salad	13	121	10·74	—	—	—	—	—
Other	3	9	33·33	—	—	—	—	—
Salad	1	16	6·25	8	414	1·93	1·18	NS
Turkey	1	7	14·29	23	509	4·52	1·22	NS
Turkey + salad	5	34	14·71	—	—	—	—	—
Total	113	725	15·59	265	4460	5·94	—	—

† From ref. [9].

n, number containing *Listeria* sp.; *N*, total number examined; *Z*-value = difference in the percentages/standard error of difference; NS, $P > 0{\cdot}05$ (not significant); ***, $P < 0{\cdot}001$ (significant at the 99·9% level); *, $P < 0{\cdot}05$ (significant at the 95% level).

(Oxoid CM862 + SR144) and stomached for 60 s at normal speed (Colworth Stomacher 400, Seward and Co. Ltd.). The broth was transferred to a honey jar and incubated at 30 °C for 24 h. After 24 and 48 h, the broth was streaked onto Oxford agar (Oxoid CM856 + SR140) and incubated at 37 °C and examined after 48 h).

Listeria counts were performed by dilution in peptone saline diluent, spreading 0·1 ml of each dilution on Oxford agar and incubating for 48 h at 37 °C.

Presumptive colonies (five) were subcultured on Columbia Agar (Oxoid) for 24 h at 37 °C and identified by catalase, motility, Gram stain, CAMP test, haemolysis and carbohydrate utilization (Rosco System, Lab M).

TVCs were performed by spiral plating and automatic colony counting [8] using plate count agar incubated at 30 °C for 72 h.

For analysis purposes the sandwiches were grouped according to the main filling and whether or not a salad component (lettuce, tomato, onion, coleslaw etc.) was included. Laboratory sample information was managed using Specimen Control System software (Microft Technology Ltd., Kew, England).

RESULTS

Table 1 shows the results of examining 725 sandwiches for *Listeria* spp. by enrichment. The sandwiches are shown by filling, with and without salad. The number and percentages of listeriae which were *L. monocytogenes* are not shown because the number of some fillings examined was small with the consequence that such figures could be misleading. Generally, sandwiches were found to be positive for *Listeria* sp. more often than their component foods in another survey [9] conducted simultaneously in the same region. For most sandwiches, the inclusion of salad slightly increased the frequency with which *Listeria* species were isolated. The small numbers of some fillings examined (e.g. bacon, turkey, cheese) means that the percentage positive may be misleading if compared directly with other fillings. This also applies to bacon, cheese, egg and seafood in the earlier study [9] where numbers of these foods sampled were considerably smaller than those of beef, chicken, ham, salad and turkey.

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Table 2. *Sandwiches* ($n = 725$) with direct counts of Listeria *species*

Filling	*L. innocua* cfu/g	*L. monocytogenes* cfu/g	TVC cfu/g
Beef + mayonnaise	100	< 100	2×10^4
Chicken + coleslaw	100	< 100	3×10^5
Egg + onion	100	< 100	3×10^5
Egg + onion	$> 2 \times 10^4$	< 100	2×10^5
Chicken	500	< 100	$> 4 \times 10^7$
Chicken	$1{\cdot}7 \times 10^3$	< 100	1×10^6
Chicken + salad	< 100	$6{\cdot}3 \times 10^4$	$> 4 \times 10^7$
Chicken + salad	< 100	100	9×10^5
Ham + tomato	< 100	200	2×10^6
Egg + bacon	< 100	$1{\cdot}4 \times 10^3$	4×10^5
Chicken	400	100	6×10^4

L. innocua and *L. monocytogenes* were the only species isolated in numbers > 100 cfu/g. These results are shown in Table 2. Eleven of 725 sandwiches examined (1·5%) contained such counts. Six sandwiches contained *L. innocua* only, 4 contained *L. monocytogenes* only, and 1 sample contained counts of both species. Six of the 11 sandwiches having raised counts contained chicken, which was found in the other study [9] to be the meat which probably represents the greatest risk to consumers. Potentially hazardous [2] levels of *L. monocytogenes* ($> 10^3$ cfu/g) were found in two sandwiches (0·3% of total examined); one chicken and salad, one egg and bacon.

DISCUSSION

Seafood sandwiches had a lower positivity rate than seafoods in the other survey [9]. This is due to most of the seafood used for sandwich filling being canned tuna/salmon, whereas most of that sampled in the other survey was restaurant/takeaway fish. For common sandwich fillings such as beef and chicken, *Listeria* sp. were found several times more frequently in sandwiches than in the foods used to fill them. The proportion of sandwiches with listeriae was found to be significantly greater than the proportion of fillings with listeriae for beef ($P < 0{\cdot}001$) and chicken ($P < 0{\cdot}05$). These results may suggest some adjustment since they were not derived from fillings actually incorporated into sandwiches, but from a large survey of miscellaneous ready-to-eat foods. It is likely that several factors are responsible. In general, smaller pieces of meat (chicken, bacon) are used for sandwich manufacture than for retail sale. The larger surface area to volume ratio of the smaller pieces tends to increase the counts per unit weight. The additional handling involved in cutting meats and sandwich assembly adds to the likelihood of contamination. Storage of fillings in cartons and hoppers during manufacture often is not temperature controlled and may facilitate the growth of bacteria. For other foods there was little evidence of a linear relationship between the sandwiches and fillings.

In this survey I found that over 15% of sandwiches contained *Listeria* sp. on enrichment, 1·5% contained the organisms on direct counting, and 0·7% of these counts were *L. monocytogenes*. An earlier survey of 433 sandwiches in Northern Ireland [5] found 13% contained *L. monocytogenes/ivanovii* on enrichment and 0·7% contained > 100 cfu/g of these potentially pathogenic [10, 11] *Listeria* spp. The majority of these sandwiches had been on display at ambient temperature despite industry guidelines recommending refrigerated storage [4, 6]. In spite of legislation aimed at better stock and temperature control, the occurrence of *Listeria* sp. was slightly higher than was found in the survey 5 years ago [5]. The percentage of sandwiches containing potentially hazardous levels of *L. monocytogenes* was similar in both surveys. However, the number of sandwiches containing *L. monocytogenes* was lower than the 28% of ambient display sandwiches found to contain *L. monocytogenes* in another survey [6].

Contamination may occur by various routes. These results suggest that contaminated component foods, especially salad vegetables and chicken, are probably the most common source. Cross-contamination from contaminated surfaces and utensils, unhygienic handling, inadequate washing and temperature abuse are also likely to be important factors. Field work conducted by this laboratory (results not shown) in commercial sandwich manufacturing premises has

shown that high TVCs may be present on finished sandwiches before leaving the factory and in spite of the use of a chlorine dip for salad ingredients. The addition of chlorine to water used for washing salad ingredients must be at a level which will kill micro-organisms without leaving a taint discernible to consumers. Such a balance is not easy to maintain. Considerable differences between foods prepared by different handlers were noted, but further work will be required to investigate these effects. As the sandwiches examined in this *Listeria* study originated from a large number of manufacturers, details of the hygienic standards of their preparation were not available. Bread has been found in this laboratory to be contaminated with *L. monocytogenes*, but the data we have collected from one bakery with problem drains are not sufficient to allow an estimate of the general prevalence. Butter and margarine are also possible sources of *Listeria* spp., although an earlier survey of dairy products in Northern Ireland [12] found these organisms to be absent from 34 butter samples examined.

High listeria counts were generally found on sandwiches which also had a high TVC (Table 2). However, TVC is not a good predictor because the correlation with listeria counts is not high, and a large proportion of sandwiches routinely have high counts of up to 10^8 cfu/g [2]. Potentially hazardous levels of *L. monocytogenes* were not found in sandwiches with total counts of less than 10^5 cfu/g, but since only two were found *in toto*, it is unsafe to assume that there are no exceptions to this trend.

Reliable information on the display temperature was not available for all samples received and therefore could not be correlated with the presence or count of *Listeria* sp. There are several reasons for this. Sampling personnel did not record temperatures for all samples, and temperatures vary considerably within refrigerated cabinets and during the day when storage is at ambient temperature. For foods of such a short shelf life (1–2 days), temperatures taken solely at the time of sampling could be misleading. Other studies have shown a correlation between storage temperature and TVC [4, 6] and it is likely that poor temperature control is a major factor in the continued occurrence of *Listeria* sp. in these products when the organisms are detected by direct count rather than on enrichment only. Nevertheless, it was decided that the absence of detailed temperature histories and time data prevented the useful analysis of these factors in the present study.

Chicken has been found to be frequently contaminated with *Listeria* sp. in a number of studies [9, 13, 14] and this was reflected in sandwiches containing chicken fillings. Three of five sandwiches containing unsatisfactory ($\geqslant 10^2$ cfu/g) or potentially hazardous ($\geqslant 10^3$ cfu/g) numbers of *L. monocytogenes* also contained chicken as their main filling. The microbiological quality of foods incorporated into sandwiches may be of relatively greater importance for foods which are known to have higher prevalences of specific pathogens. For other sandwich fillings, the general hygiene of preparation may be a more important factor. Further attention to this is necessary in the poultry industry.

The finding that 0·3% of sandwiches contain potentially hazardous levels of *L. monocytogenes* gives insight into the risk of contracting listeriosis and may be of use in the compilation of microbiological guidelines. The frequency of contamination in sandwiches (2/725, $< 0{\cdot}3\%$) did not differ significantly ($P > 0{\cdot}05$) from the ready-to-eat foods generally (14/8360, $< 0{\cdot}2\%$) [9]. Such a level of contamination would be unacceptable if the organism were salmonella. Unlike salmonella which can cause serious illness in healthy individuals, *L. monocytogenes* appears to be a significant hazard principally amongst the immunocompromised. The absence of listeriosis cases despite the large number of commercially-made sandwiches consumed suggests that the risk of disease from *L. monocytogenes* is much lower than from salmonella. This is largely due to the immune competence of the general population. A separate risk assessment would be required for immunocompromised groups. The likelihood of *consuming* a potentially infectious dose of *L. monocytogenes* must be distinguished from the likelihood of *contracting* foodborne listeriosis. Practically, this may be very difficult.

A zero tolerance level for *L. monocytogenes* gives a high assurance of safety but may result in the condemnation of excessive quantities of food and may not be necessary if a lower level of safety is acceptable. In a recent judgement on Lanark Blue cheese [15] the Sheriff indicated that the food was not unfit despite high levels of *L. monocytogenes* largely because there was no epidemiological evidence of illness related to consumption of the cheese. Increased use of subtyping may be necessary when high numbers are isolated to relate subtypes of *L. monocytogenes* to their pathogenic potential, for legal, if not scientific reasons. In foods intended for consumption by the general

population such a balance between safety assurance and overcautious enforcement is difficult to strike because of the complexities surrounding the infectious dose. The Lanark Blue judgement may have been convenient for the food industry, at least in the short term, but shifting the emphasis from safety to complacency may prove not to be in the general public interest in the longer term. Deaths resulting from infection with this organism will be likely to swing opinion once again towards zero tolerance. At present it is important to learn more about the pathogenicity of *L. monocytogenes* subtypes and epidemiological risk factors aiming at more quantitative risk assessment.

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