The microbiology of sausages from some retail supermarkets in the Dublin area

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Introduction

Fresh sausages as they are sold in Ireland are generally similar to the British version. As such they are an emulsion of meat, fat, rusk and seasonings, contained in a man-made or natural casing. Comminuted meat products of this kind tend to have a shorter shelf-life. In circumstances where the initial contamination levels are high the shelf-life of the product will be even further reduced. Quite apart from considerations regarding spoilage of sausages it has also been shown that in a survey carried out in Britain by Roberts *et al.*, (1975) that *Salmonella* spp. were present in 29.7% of all packets of sausages examined. The potential of these products as sources of food poisoning bacteria is apparent.

A preliminary survey was carried out in the Dublin area on packs of sausages on sale in some retail supermarkets. The purpose was to establish some baseline data on the spoilage potential of a number of brands of sausages and their possible public health risk in terms of the presence of *Salmonella*.

Materials and Methods

A microbiological and chemical assessment was carried out on sixteen different brands of pork sausages and one each of beef and turkey. Each brand was sampled 4 times at weekly intervals by purchasing two half-pound (228 g) packages. Fourteen of the samples were in cellophane pre-packs and two were sold loose. The pre-packs were taken from refrigerated retail display cabinets and on each sampling occasion one pack was taken from the top and one from the bottom of the cabinet. Sampling was on Thursday or Friday of each week. These days were chosen to ensure that packs were from the most recent production runs. The packs were transported to the laboratory in refrigerated containers within 2 hours of purchase. They were kept in a chill room at 1°C until the following Monday or Tuesday, a period of 3 - 4 days.

Microbiological tests of the samples were carried out as follows. Sausages from the two half-pound pre-packs in each brand were combined and a sample was aseptically removed from the centre of 3 - 4 sausages using a sterile scalpel. Sausages sold loose were similarly treated. A composite 10 g sample was placed in a sterile plastic bag,

together with 90 ml of quarter strength Ringer's solution + 0.1% added peptone, and blended for 1 minute using a Colworth stomacher.

Pre-dried plates of Oxoid Tryptone Glucose Yeast Agar (TGYA) were quartered and surface inoculated in duplicate with 0.025 ml amounts of the neat solution or successive tenfold dilutions of this. The pH of the medium was adjusted to 7.0 - 7.2. Lactobacilli and other catalase negative organisms were enumerated in the same way on the MRS medium of de Man *et al.*, (1960), as were the yeasts and moulds using Oxoid malt extract agar (MEA) to which sterile 10% lactic acid was added to bring the pH to 3.5. The presumptive Pseudomonads were estimated using the cephaloridine, fucidin, cetrimide (CFC) medium of Mead and Adams (1977). Presumptive *Brochothrix thermosphacta* was isolated using the thallous acetate, actidione agar medium (STAA) of Gardner (1966). All plates were incubated at 25°C for 3 days.

Escherichia coli was isolated by filtering 1.0 ml of the macerate through an Oxoid membrane filter. The membrane was incubated for 2 h at 37°C on a pad containing Oxoid resuscitation broth. The membrane was then transferred to a second pad containing Oxoid McConkey broth and incubated overnight at 44°C in a waterbath.

Two 25 g samples were taken as described above and stomached in sterile plastic bags in 50 ml of quarter-strength Ringers solution + 0.1% added peptone. The contents of one bag were added to 50 ml of Oxoid Tetrathionate broth and incubated at 37°C for 48 h. The second sample was added to 50 ml of Oxoid Mannitol Selenite broth and incubated at 44°C for 48 h. Plates of Oxoid Brilliant Green Agar (BGA) were streaked from the contents of the two media and pink colonies, typical of *Salmonella*, were picked off for further identification.

The presence of preservative in the sausages, as sodium metabisulphite, was estimated as sulphur dioxide (SO_2) on 100g samples, using the method described by Pearson (1970). The pH of all the samples was taken using an Orion Model 221 meter, with a combined glass probe (no. 91-63-00). Each result is the mean of five readings.

An analysis of variance was carried out to test the difference between the 18 brands and the four trials. (The interaction term was used for "error").

Results

There was a large variation between brands as shown by the total viable counts (TGYA) and the different selective media (Table 1). Although only one brand of beef and turkey sausages was examined, the results obtained for these did not suggest that they were different from the pork brands.

TABLE 1: Mean numbers of bacteria (log_{10}/g) from plates of TGYA, STAA, MEA, MRS and CFC, sulphur dioxide (SO_2) and pH values, from eighteen brands of sausages.

	Bacterial numbers (log_{10}/g) on different selective media						
Brand	TGYA	STAA	MRS	MEA	CFC	SO_2 (µg/g)	pH
A	6.64	6.16	4.96	5.36	4.24	351	6.17
B	6.77	4.22	6.22	5.26	4.28	340	6.35
C	6.83	6.32	5.49	5.16	4.67	209	6.20
D	6.88	5.56	5.37	5.64	4.70	413	6.37
E	7.02	7.07	5.35	5.44	3.97	338	6.14
F	7.04	5.59	6.24	6.26	4.94	372	6.07
G	7.21	5.43	5.87	5.95	4.82	334	6.28
H	7.41	7.45	5.77	5.49	5.09	328	5.97
I	7.48	7.09	5.04	5.03	4.26	354	6.28
J	7.63	7.06	3.82	4.73	3.96	314	6.28
K	8.65	7.96	5.74	5.66	5.66	307	6.19
L	8.70	8.43	5.15	4.60	4.81	640	6.16
M	8.79	7.98	6.65	6.13	5.44	382	6.05
N	9.11	8.41	7.30	5.46	4.06	320	5.74
O	9.16	8.51	5.70	5.82	5.95	143	5.97
P	9.47	8.96	6.43	5.85	5.31	164	5.99
Q (beef)	7.57	6.80	6.72	6.36	5.70	358	6.30
R (turkey)	8.57	6.84	4.72	4.77	4.67	325	6.42
F-test	4.37***	8.15***	5.09***	2.73***	1.81*	15.6***	3.39***

Significance level *P<.05 ***P<.001

In general *B. thermosphacta* was the predominant spoilage organism present, with the exception of brands B, D, F, G and Q. In the case of brand D the yeast and mould counts, (MEA) were marginally above those on STAA. On brands B and Q the lactic flora (MRS) was dominant, while the MEA and MRS counts were greatest and about equal on brands F and G. The presumptive *Pseudomonas* count always accounted for the smallest portion of the spoilage flora.

The preservative in the sausages varied from 143 to 640 µg/g, with a mean value of 333 µg/g. There was only a small variation in pH values where the mean was 6.16 (range 6.42 - 5.74). There was no relationship between either of these two parameters and the spoilage flora.

In Table 2 the bacteria on the different selective media are expressed as a percentage of the total count on TGYA. The data emphasise the predominance of *B. thermosphacta* as the main spoilage organism, and the insignificant numbers of Pseudomonads present. Of particular significance is the fact that the selective media in most instances, accounted for only a small proportion of the counts on TGYA. The spoilage flora comprised on average only about 30 - 40% of the total on TGYA plates and the remainder was composed of other organisms, mostly saprophytic. In the two brands (E, H) the STAA counts alone exceeded the numbers on TGYA, suggesting that the latter medium did not always recover the total numbers of bacteria present on the meat.

Faecal contamination of the sausages, as measured by the presence of *E. coli* in the samples, was very low (Table 3). As for the other bacterial counts there was no relationship between the presence of *E. coli* and the level of SO_2 in the sausage meat. There was a complete absence of *Salmonella* from all the brands tested.

Discussion

The total counts and those on the selective media were considered to represent the initial flora of the sausages examined in the present study. Although a period of some days elapsed before the samples were examined, the very low storage temperature would have precluded any significant increase in microbial growth. In their work, Dyett and Shelley (1962) stored 1 lb packs of pork sausages at 3.5°C and there was no increase in the total counts at 22°C over an 8 day period. A similar observation was made by Dowdell and Board (1968) on pork and beef sausages and they stated that "the genesis of the microbial association can be detected shortly after the preparation of the sausages, short term storage does not appear to permit significant microbial growth".

According to Dyett and Shelley (1966) the total bacterial count at 22°C is the most useful indicator of organoleptic spoilage. Dowdell and Board (1968) indicated that the standard of British sausages, based

TABLE 2: Total numbers of organisms on plates of STAA, MEA, MRS and CFC, expressed as a percentage of the total count on TGYA

Brand	Media				
	STAA	MRS	MEA	CFC	Percentage (%) of total flora accounted for
A	33.11	2.10	5.25	0.40	40.86
B	0.28	28.18	3.09	0.32	31.87
C	31.00	4.57	2.14	0.69	38.40
D	4.79	0.01	5.75	0.01	10.56
E	112.20	2.14	2.63	0.09	117.06
F	3.55	15.85	16.60	0.79	36.79
G	1.66	4.57	5.50	0.41	12.14
H	109.65	2.29	1.20	0.48	113.62
I	40.74	0.36	0.36	0.06	41.52
J	26.92	0.02	0.01	0.02	26.97
K	20.42	0.12	0.10	0.10	20.74
L	53.70	0.03	0.01	0.01	53.75
M	15.49	0.72	0.22	0.05	16.48
N	19.95	1.55	0.02	0.01	21.53
O	22.39	0.04	0.05	0.06	22.54
P	30.90	0.09	0.02	0.01	31.02
Q (beef)	16.98	14.13	6.17	1.35	38.63
R (turkey)	1.86	0.01	0.02	0.01	1.90
Mean	30.31	4.32	2.73	0.27	37.58

TABLE 3: Numbers of *E. coli*/g isolated from eighteen brands of sausages

Brand	*E. coli* counts ($\log_{10}$/g)
A	0.25
B	0.25
C	1.27
D	1.10
E	0.0
F	1.45
G	1.89
H	0.33
I	2.58
J	1.65
K	2.03
L	0.45
M	1.15
N	0.43
O	0.50
P	0.65
Q (beef)	0.83
R (turkey)	1.48
Mean	1.02

on the total count, was between $\log_{10}$ 5.0 and $\log_{10}$ 8.7/g. In the present study almost half of the brands examined were at or above the higher value. Goldenberg (1964) states that sausages with a total count in excess of $\log_{10}$ 6.7/g are of 'suspicious' bacteriological quality. All the samples examined in the present study would at least be in this category.

The results of Dowdell and Board (1968) established that the most common contaminant of pork and beef sausages was *B. thermosphacta*. While this was also noted in the present study it was observed that a large proportion of the total flora was unaccounted for. It is assumed that the remainder of the flora is composed mainly from species of *Bacillus* and micrococci (Dyett and Shelley, 1966). The four 'microbiological types' of sausages described by Dowdell and Board (1968) were not apparent from the present work. Dyett and Shelley (1962) state that the legal limit for incorporating sulphite in sausages is 450 µg/g and that most manufacturers use the maximum concentration in their products. The concentration used in the present study would tend to suggest that the majority of manufacturers would at least approach the 350 µg/g level, giving adequate protection to their product. In only one case was the level greatly in excess of the 450 µg/g legal limit. The low numbers of Pseudomonads may partly be accounted for in this way, since gram negative organisms are most inhibited by this preservative (Dyett and Shelley, 1966).

In Britain and the United States 29.7 and 23% respectively of pork sausages were found to harbour salmonellae (Roberts *et al.*, 1975; Galton *et al.*, 1954). These results are in sharp contrast to those in the present study where *Salmonella* was completely absent. This result may however, be misleading, since the sample numbers in the present work were very small (72) compared to the very large numbers (3309 and 217) in the other surveys. Further work over a longer time period is needed to confirm this point.

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