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The microbiology of selected retail food products with an evaluation of viable counting methods

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SUMMARY

In an inter-laboratory survey, the pour plate, surface spread, agar.droplet and spiral plate methods were used in parallel with the surface drop method for enumeration of micro-organisms in foods. Good agreement was obtained between all surface methods of enumeration, but there was poor agreement between molten agar methods and the surface drop method.

A total of 1143 samples of food that were ready for consumption at the point of retail sale were examined. Eight types of food products were chosen: meat pasties, sausage rolls, real-cream slices, synthetic-cream slices, mayonnaise-based coleslaws, faggots, patés and continental sausages. The results of this survey suggest that the upper limit for an acceptable viable count should vary according to the food product. Salmonellae were not isolated on any occasion. Potentially harmful organisms were not isolated at levels expected to constitute a public health hazard.

Information concerning the nature of the product, the total viable count, the presence or absence of pathogenic, toxigenic or indicator organisms, the spectrum of the bacterial flora and the relative predominance of each organism must all be considered when assessing the microbiological acceptability of retail 'ready to eat' products.

INTRODUCTION

Analysis of food samples in laboratories of the Public Health Laboratory Service (PHLS) is carried out at the request of the environmental health departments of local authorities, usually as a result of a complaint or food poisoning incident, or as part of an investigation into standards of storage, hygiene and handling practices at a retail outlet. In order to interpret the results of these microbiological investigations it is necessary to have some knowledge of acceptable standards. Published microbiological limits generally refer to manufacturers' specifications applied to the food product as it comes off the production line or before it has reached the retailer, for example the recommendations made by the International

Committee on Microbiological Specification for Foods (ICMSF) (Thatcher & Clark, 1974). Some states in the USA have formulated guidelines for certain foods at the point of retail sale (Wehr, 1978). In this country the bacteriology of cooked meats available to the consumer has been investigated (Barrell & Watkinson, 1981), but little other information concerning food at the point of retail sale has been published.

Microbiological examination of foods which have been cooked or are otherwise 'ready to eat' usually involves the enumeration of mesophilic aerobic micro-organisms (total viable count, TVC) and examination for the presence of certain enteropathogenic, toxigenic and indicator organisms. Traditional methods of performing viable counts are the pour plate, surface spread plate and modified Miles and Misra surface drop plate methods (Thatcher & Clark, 1968). Recent developments have introduced some mechanization to enumeration techniques, and the agar droplet method (Sharpe & Kilsby, 1971; Sharpe *et al.* 1972) and the spiral plate method (Gilchrist *et al.* 1973) have become increasingly popular.

Four neighbouring public health laboratories have taken part in a survey of the microbiological quality of certain food items ready for consumption at the point of retail sale. At the same time, the pour plate, surface spread plate, agar droplet and spiral plate methods were compared with the surface drop method for enumeration of micro-organisms in foods.

MATERIALS AND METHODS

Sampling

Samples of food were obtained from retail outlets by members of the environmental health departments. Samples were transported to the local laboratory and examined on the day of purchase.

Cooked meat pasties, sausage rolls, real and synthetic cream slices, patés, cooked faggots, mayonnaise-based coleslaws and continental sausages were examined.

Microbiological methods of examination

Preparation of samples

For most food items, a representative sample of the product was taken, but for continental sausages core samples were used. At least 10 g of food sample was weighed, sufficient 0·1 % peptone water (Oxoid) added to form a 1/10 dilution, and the sample blended in a Colworth 'Stomacher 400' (A. J. Seward, Bury St Edmunds, Suffolk). This 1/10 suspension was used for all further dilutions. Serial decimal dilutions were made in 0·1 % peptone water for use in the pour plate, surface spread and surface drop plate methods of enumeration. Decimal dilutions were made in cooled, molten agar for the droplet method. A 1/5000 dilution in 0·1 % peptone water was made using the spiral plater (see below) for use in the spiral plate method.

Total viable counts (TVC)

Total viable counts were performed using a diagnostic sensitivity testing agar for convenience (iso-sensitest agar, Oxoid; diagnostic sensitivity test agar, Oxoid; or sensitest agar, Gibco). All plates were incubated at 30 °C for 2 days.

Each laboratory used

(1) Surface drop method (Miles & Misra, 1938) modified according to Thatcher & Clark (1968).

In addition, every laboratory also performed a total viable count by an alternative method of their choice. These alternative methods were:

(2) Pour plate (Thatcher & Clark, 1968).

(3) Surface spread plate (Thatcher & Clark, 1968).

(4) Agar droplet (Sharpe & Kilsby, 1971) using a Colworth 'Droplette' machine available from A. J. Seward, Bury St Edmunds, Suffolk.

(5) Spiral plate (Gilchrist *et al.* 1973) using a 'Spiral Plater' available from Don Whitley Scientific, Shipley, West Yorkshire. Colonies on the spiral plate were counted using a calibrated grid or an Exotech Laser Bacteria Colony Counter, available from Don Whitley Scientific.

Detection of organisms

Staphylococcus aureus

0·5 ml of the 1/10 homogenate was applied to the surface of two Baird-Parker agar plates (Baird-Parker, 1962) and the plates incubated at 37 °C for 2 days. Typical black, shiny colonies surrounded by a zone of opalescence or clearing were counted, subcultured, and confirmed as *Staph. aureus* by testing for coagulase and/or deoxyribonuclease production.

Bacillus cereus (*presumptive*)

0·5 ml of the 1/10 homogenate was applied to the surface of two Columbia agar plates (Oxoid) containing 5% egg yolk, and the plates incubated at 37 °C overnight. Typical colonies surrounded by a zone of opalescence were counted.

Clostridium perfringens

0·5 ml of the 1/10 homogenate was applied to the surface of two Columbia agar plates containing 5% egg yolk and 100 μg/ml neomycin sulphate. The plates were incubated anaerobically at 37 °C overnight, and colonies surrounded by a zone of opalescence were counted. Colonies were confirmed as *Cl. perfringens* using the litmus milk reaction or by testing for the inhibition of production of the zone of opalescence by *Cl. perfringens* antitoxin A.

Escherichia coli

0·5 ml of the 1/10 homogenate was applied to the surface of two violet-red bile agar (Oxoid), MacConkey agar (Oxoid) or Teepol lactose agar plates, and the plates incubated overnight at 37 °C. Lactose-fermenting colonies were counted, and confirmed as *E. coli* by further testing for indole production in peptone water and acid and gas production from lactose at 44 °C.

Yeasts

0·5 ml of the 1/10 homogenate was applied to the surface of two Sabouraud dextrose agar plates (Oxoid), containing 100 μg/ml gentamicin when necessary to prevent overgrowth by gram-negative bacilli. The plates were incubated at 30 °C for at least 2 days. Suspect colonies were confirmed by Gram stain.

Table 1. *Total viable counts (TVC) obtained from eight food products by the surface-drop method*

Food product	No. of samples	Log_{10} TVC/g Mean	Log_{10} TVC/g Range
Meat pastie	123	< 3·00*	< 2·40–7·11
Sausage roll	118	< 3·00*	< 2·40–5·34
Real-cream slice	92	5·57	2·40–8·70
Synthetic-cream slice	101	3·78	< 2·40–7·00
Coleslaw	185	3·20	< 2·40–9·28
Faggot	188	4·20	< 2·40–9·00
Paté	187	5·00	2·40–10·00
Continental sausage	149	5·40	< 2·40–9·63

* 81 % of meat pasties and sausage rolls had an undetectable viable count (< log_{10} 2·40) by the surface-drop method.

Salmonella

50 ml of the 1/10 homogenate was added to an equal volume of double-strength selenite solution and incubated at 37 °C for 2 days. The selenite broth was then subcultured to the selective media currently used by each laboratory for detection of salmonellae. Suspect colonies were subjected to biochemical and serological testing.

Other organisms

Other organisms were identified from total count plates using standard techniques.

RESULTS

Total viable counts

During a period of 18 months, 1143 samples of food were tested. Table 1 shows the range and overall mean colony count and Fig. 1 illustrates the distribution of counts for each type of food product obtained by the surface-drop method. Table 2 shows the number of products examined by each laboratory, and Table 3 the mean colony counts obtained by each laboratory for each product. Differences in the distribution of total viable count did not appear to be related to laboratory technique, but could be attributed to a limited number of producers retailing in certain areas.

Four hundred and fourteen of these products were sampled by the spiral plate method of enumeration, 184 by the surface spread method, 308 by the pour plate method and 237 by the droplet method. Regression and correlation coefficients are shown in Table 4 and lines of regression illustrated in Fig. 2. The graph indicates that the spiral plate results were slightly higher than by traditional surface-enumeration methods. From products possessing a detectable viable count, there was a difference of more than one log_{10} cycle in only 3·5 % of pairs of surface drop/spiral plate counts and 7·4 % of pairs of surface drop/surface spread counts. The regression line for pour plate results shows slightly lower values than by surface enumeration methods, but 20·5 % of pairs of surface drop/pour plate

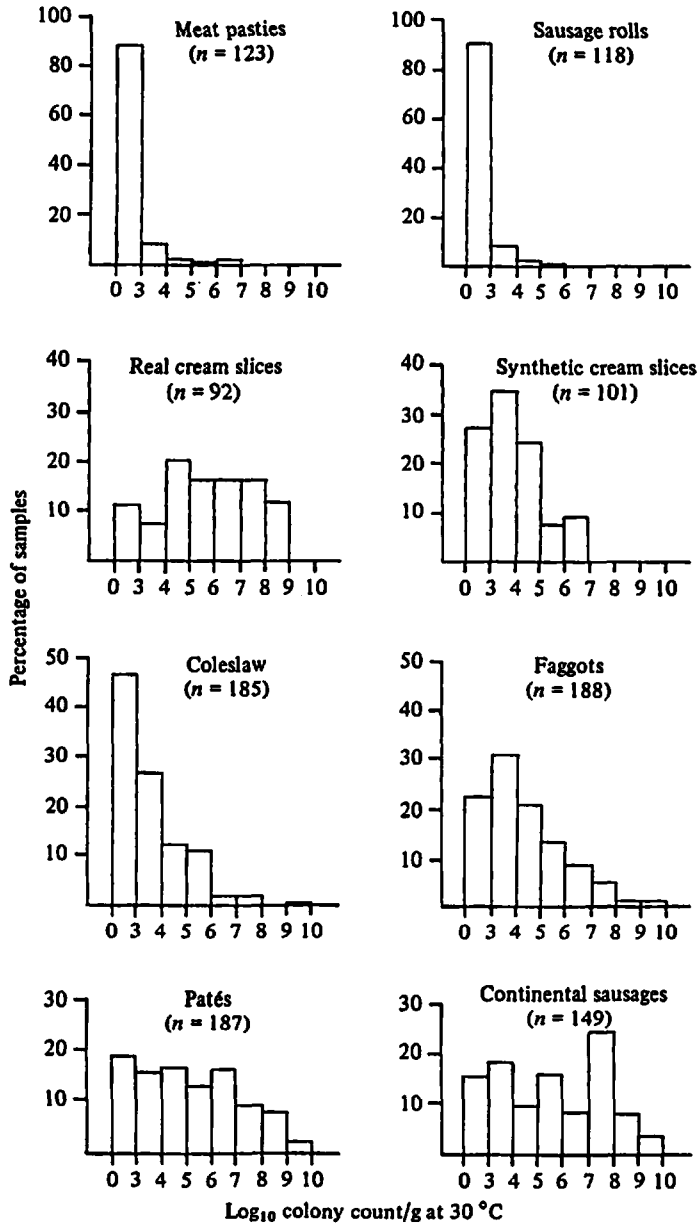

Fig. 1. The distribution of colony counts at 30 °C in eight 'ready to eat' food products.

counts differed by more than one $\log_{10}$ cycle. Agar droplet results were inconsistent; when viable counts were less than 10^4/g, values obtained by the droplet count were greater than by the surface-drop method, but this was reversed when viable counts exceeded 10^4/g. Furthermore, only 73% of pairs of surface drop/droplet counts were within one $\log_{10}$ cycle of each other.

Table 2. *Number of samples examined by each laboratory*

	Laboratory				
Food product	A	B	C	D	Total
Meat pastie	41	18	45	19	123
Sausage roll	42	18	43	15	118
Real-cream slice	38	10	23	21	92
Synthetic-cream slice	24	22	32	23	101
Coleslaw	61	31	44	49	185
Faggot	58	29	45	56	188
Paté	72	40	57	18	187
Continental sausage	78	16	19	36	149

Table 3. *Mean colony counts obtained by each laboratory for eight food products (mean $\log_{10}$ colony count/g)*

	Laboratory			
Food product	A	B	C	D
Meat pastie	< 3·00	< 3·00	< 3·00	< 3·00
Sausage roll	< 3·00	< 3·00	< 3·00	< 3·00
Real-cream slice	5·83	5·79	3·93	6·78
Synthetic-cream slice	4·03	3·72	3·88	3·44
Coleslaw	3·28	4·00	3·62	2·52
Faggot	3·63	4·65	4·00	4·75
Paté	4·71	4·32	6·02	4·43
Continental sausage	5·51	6·35	4·25	5·33

Table 4. *Regression and correlation coefficients obtained by the surface drop method on corresponding results of four other methods of bacterial enumeration*

Reference method	*v.* Test method	Regression coefficient (β)	Intercept (α)	Standard error, 95% c.i. (S.E. β)	Multiple correlation coefficient (r)
Surface drop	Spiral plate	1·052	−0·114	0·033	0·974
Surface drop	Surface spread	1·014	−0·116	0·047	0·966
Surface drop	Pour plate	0·962	0·051	0·085	0·852
Surface drop	Agar droplet	0·630	1·595	0·108	0·731

Table 5. *Incidence of specified organisms in eight food products (organisms detected in 0·1 g by direct surface plating)*

	Percentage occurrence							
	Meat pastie	Sausage roll	Real-cream slice	Synthetic-cream slice	Cole-slaw	Cooked faggot	Paté	Conti-nental sausage
Staph. aureus	4·9	7·6	25·0	11·9	6·5	6·4	1·6	14·8
Cl. perfringens	4·9	3·4	NT	NT	NT	4·3	1·1	6·0
B. cereus	3·3	0·8	16·3	4·0	13·5	11·7	NT	1·0
E. coli	0·0	2·4	17·4	7·9	1·1	5·3	3·7	0·0
Yeasts	NT	NT	50·0	51·9	27·3	NT	NT	22·3

NT = not tested.

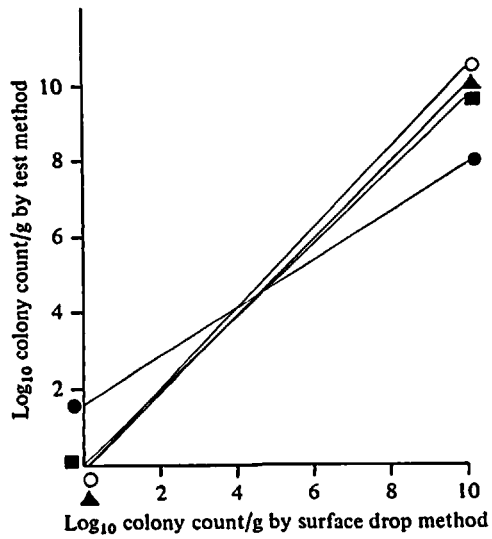

Fig. 2. Lines of regression for bacterial enumeration methods. Regression of colony count data from the surface-drop method (X) on corresponding results by (○), spiral plate method ($Y = 1{\cdot}052X - 0{\cdot}114$); (▲), surface spread method ($Y = 1{\cdot}014X - 0{\cdot}116$); (■), pour-plate method ($Y = 0{\cdot}962X + 0{\cdot}051$); (●), agar droplet method ($Y = 0{\cdot}63X + 1{\cdot}595$).

Table 6. *Quantitative estimates of specified organisms in eight food products*

	No. of products tested	No. (and %) of products containing organism	Log_{10} count/g 1·00–1·99	2·00–2·99	3·00–3·99	4·00–4·99	5·00–5·99	6·00–6·99	Presence in 0·1 g*
Staph. aureus	1143	99 (8·7)	72	19	5	—	—	—	3
Cl. perfringens	765	29 (3·8)	26	2	1	—	—	—	—
B. cereus	904	72 (8·0)	54	16	—	1	—	—	1
E. coli,	985	44 (4·5)	17	11	5	—	1	2	8
Yeasts	430	149 (34·7)	45	44	31	10	1	5	13

* Detected, but not enumerated.

Detection of organisms

Salmonella spp. were not detected in any sample. The incidence of *Staph. aureus*, *Cl. perfringens*, *B. cereus*, *E. coli* and yeasts in the different food products is shown in Table 5, and quantitative estimates of these organisms are shown in Table 6. *Staph. aureus*, *Cl. perfringens* and *B. cereus* were infrequently isolated and rarely occurred at levels greater than 1000/g, whilst *E. coli* only occurred regularly in real-cream slices, but yeasts were commonly found in all products in which they were sought. No correlation was found between a high TVC and the presence of indicator organisms.

The bacterial flora of products with a detectable viable count was recorded from the total count plate. The results are summarized in Table 7, together with indications as to the origin of the bacteria.

Table 7. *The bacterial flora of food products*

Food product	Organisms	Comment
Meat pasties, sausage rolls	Mainly staphylococci and aerobic spore bearers; occasionally micrococci, enterococci and gram-negative bacilli	Organisms introduced by handling and open storage.
Patés, faggots, continental sausage	When TVC $< 10^6$/g: staphylococci, micrococci, aerobic spore bearers, and occasional gram-negative bacilli When TVC $> 10^6$/g: lactobacilli and streptococci	Organisms introduced by handling and storage. Mode of production allows survival of these organisms. Inadequate refrigeration allows growth.
Coleslaws	Mixed flora, predominantly gram-positive cocci, aerobic spore formers and yeasts When TVC $> 10^5$/g: predominantly yeasts and lactobacilli	Organisms introduced with raw ingredients and handling. Acidity favours growth of yeasts. Inadequate refrigeration allows growth of lactobacilli.
Real-cream slices	Predominantly gram-negative bacilli; also yeasts and gram-positive cocci	Gram-negative bacilli introduced by cream.
Synthetic-cream slices	Predominantly yeasts, streptococci, staphylococci and gram-negative bacilli	Organisms originate from ingredients and handling. Storage at ambient temperature allows growth.

DISCUSSION

Previous comparison between methods of enumeration of micro-organisms have been carried out under strictly controlled conditions on pure cultures of bacteria, spore suspensions, or relatively small numbers of food samples (Jarvis, Lach & Wood, 1977; Hedges, Shannon & Hobbs, 1978; Kramer & Gilbert, 1978, Kramer, Kendall & Gilbert, 1979). These workers showed a good correlation between the methods tested. Best correlation between methods is achieved when all counting methods are performed by the same operator. Our study was carried out in four different laboratories under 'normal' conditions over a period of 18 months, and reflects variations such as different levels of technical expertise and the possibility of errors being introduced during transcription and calculation. Interlaboratory variations seen in Table 3 could be attributed to limited numbers of producers retailing in some locations, and a collaborative survey such as this which covers a relatively large geographical area helps to overcome bias in results obtained by individual laboratories due to these factors.

Using the surface drop technique as the reference method, there was good correlation with results from the spiral plate and surface spread methods. The pour plate method does not correlate as well with the surface drop technique, gives lower results and a greater variation between pairs of counts. This is of particular

interest as it is the method favoured by European and American workers for quality control of food products. The agar droplet method of enumeration was unsatisfactory in this study. It gave considerable and inconsistent variation from results obtained by the surface drop method, and counting was found difficult by the operators, due to the presence of food particles.

We recommend surface methods of enumeration for monitoring viable counts in food products. The spiral plate method is particularly recommended for its savings in labour and materials. Inconsistencies in the results obtained with the agar droplet method suggest that this is not suitable for use in the examination of foods.

An arbitrary level of 10^6/g is frequently used as the upper limit for acceptable viable counts for food products that are ready for consumption (Hobbs & Gilbert, 1970). However, Bassett, Kurtz & Moore (1978) found no relationship between colony counts and the wholesomeness of cooked meats in relation to their appearance, smell and palatability, and a microbiological evaluation of delicatessen meats (Tiwari & Kadis, 1981) obtained mean aerobic plate counts greater than 10^6/g for seven out of 17 types of product.

Analysis of the distribution of viable counts obtained in this study (Fig. 1) suggests that the acceptable upper limit for viable counts varies considerably from product to product. Most samples of meat pasties and sausage rolls examined were found to comply with end-point specifications set by the food industry for meat pies (Goldenberg, 1964; Davis, 1969); 90% of samples had a viable count of less than 10^3/g. A count in excess of 10^4/g might suggest that the product had been subjected to prolonged storage at ambient temperature. The type of cream used to fill cream slices affects the count for this product. Of the slices filled with real cream, 45% possessed a viable count exceeding 10^6/g, and viable counts of 10^9/g were found without any apparent detriment to the wholesomeness of the product. By comparison, most synthetic-cream slices (80%) had viable counts less than 10^4/g, and counts above 10^6/g were uncommon. The mean colony count of mayonnaise-based coleslaws is low (10^3/g); 96% of these products had a viable count less than 10^6/g. A higher count would suggest inadequate refrigeration or poor stock rotation. Of cooked faggots, 85% had viable counts less than 10^6/g. These products are usually reheated following purchase and a further reduction in count is likely prior to consumption. 35% of patés and 42% of continental sausage samples possessed total counts exceeding 10^6/g. These results are similar to those obtained by Barrell & Watkinson (1981) and Tiwari & Kadis (1981).

Tables 5 and 6 indicate that the potentially enterotoxigenic organisms *Staph. aureus*, *Cl. perfringens* and *B. cereus* occurred infrequently and at low levels. These organisms usually need to be present in large numbers ($> 10^6$/g) to be hazardous to the consumer. If levels of these organisms exceed 100/g, further investigation may be indicated. If *Staph. aureus* is present, the hygiene of food-handlers and equipment is suspect, whilst if spore-formers are present, the control of cooling processes following cooking merits investigation.

Although the overall occurrence of *E. coli* was low, over half the isolations were at levels greater than 100/g. Isolation from real-cream slices occurred frequently and more than 10% of these samples contained *E. coli* $>$ 100/g. This suggests that post-pasteurization contamination occurs at some dairy premises.

Yeasts were isolated in relatively high numbers from over one-third of all samples examined for their presence. Many of these organisms are psychrotrophic and are able to grow at low pH. They may be introduced by the raw materials, the environment, or the preserving fluid – e.g. vegetables in coleslaws, bakeries or pickling brines. Their presence in large numbers represents a spoilage problem, but has little public health significance.

By examining fairly large numbers of each type of product, it was possible to make certain generalizations about their bacterial populations (Table 7). The lactic acid bacteria found in high numbers in patés, faggots and continental sausage samples are part of the normal flora of meat products. Some strains are actually used during fermentation processes. The mode of production of patés and, in particular, continental sausages allows survival of these organisms. Certain strains of lactic acid bacteria can grow at temperatures as low as 6 °C, and unless strict temperature control is maintained they may proliferate. Their presence is, however, harmless to the consumer. When interpreting total count results, it is important to distinguish between these lactic acid bacteria and other groups of organisms such as gram-negative bacilli or aerobic spore formers, whose presence in large numbers should be considered abnormal.

Results obtained from this collaborative survey have shown that the nature of the product, the total viable count, the presence or absence of enteropathogenic or indicator organisms, the spectrum of the bacterial flora and the relative predominance of each organism are all factors to be considered in assessing the microbiological acceptability of 'ready to eat' foods.

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