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The quantitative bacteriology of some commercial bivalve shellfish entering British markets

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SUMMARY

Incidents of non-specific illness associated with the consumption of oysters have highlighted the lack of published information on the bacteriology of shellfish suitable for consumption. Investigations showed that the majority of molluscan shellfish entering English markets conform to the accepted standard of less than 5 *Escherichia coli*/ml. tissue. The numbers of *E. coli* were related to the sanitary quality of the growing area but no relation could be established between numbers of *E. coli* and coliforms, faecal streptococci or *Clostridium welchii*. The numbers of non-specific bacteria varied considerably but shellfish from sources associated with non-specific illness yielded relatively high counts at 37° C. The results showed that there was no justification for a standard based on total plate counts, which often exceeded 10^6/g. Such a standard would have to be coupled with spoilage or the incidence of non-specific illness. The relation between the numbers of non-specific bacteria growing at 20 and 37° C. appears to be a useful measure for assessing the likelihood that raw shellfish are a public health risk.

INTRODUCTION

Investigations into the possible causes of non-specific illness following the consumption of raw oysters (Preston, 1968; Gunn & Rowlands, 1969; Ayres, 1971) have highlighted considerable gaps in our knowledge of the normal bacteriological flora of shellfish. In the United Kingdom suitability for consumption is based largely upon the presence of *Escherichia coli*, using the standards suggested by Sherwood & Thomson (1953) which, although successfully applied by local public health authorities, are not statutory. At the present time, there are few generally accepted guidelines which can be used to assess the significance of other bacteria present in molluscan shellfish. In an attempt to overcome this deficiency, samples of molluscan bivalve shellfish taken from the major producers and markets in England were subjected to bacteriological analysis for *E. coli*, coliforms, faecal streptococci, *Clostridium welchii* and bacteria growing on non-selective media (referred to here as the total plate count).

Table 1. *Source, species and number of shellfish samples examined*

Source	Species	No. of samples examined
East coast		
A	European flat oysters	16
B	European flat oysters	18
	Portuguese oysters	6
C	European flat oysters	3
	Portuguese oysters	6
	Hard clams	6
South coast	European flat oysters	8
South-west coast		
A	European flat oysters	12
B	European flat oysters	25
C	European flat oysters	11
D	European flat oysters	19
E	Mussels	4
F	Mussels	7
Coast of Wales		
A	Mussels	9
B	Mussels	4
Coast of Ireland		
	Mussels	13
	European flat oysters	1
Coast of Scotland	European flat oysters	1
Total		169

MATERIALS AND METHODS

Samples of the European flat oyster (*Ostrea edulis*), Portuguese oyster (*Crassostrea angulata*), mussel (*Mytilus edulis*) and hard clam (*Mercenaria mercenaria*) were obtained from producers or wholesalers. To simulate as closely as possible the conditions in which shellfish would be received for consumption, samples were sent to the laboratory by post, where they were examined the day following dispatch. Sampling began in January 1970, corresponding to approximately halfway through the season for flat oysters (September–March) and continued until the season closed. To ensure representative sampling a further series began in the following September and continued until the end of December. Geographic origins and numbers of samples examined of each species are shown in Table 1.

Bacteriological examination

Ten individual animals constituting a sample were thoroughly scrubbed to remove mud and epifauna from the shells before examination. Each sample was opened aseptically into a sterile weighed container and diluted with an equal volume of sterile 0·1 % (w/v) peptone water before maceration for 15 sec. at half speed (6000 rev./min.) in a MSE 'Atomix' stainless-steel jar. A further volume of diluent was added after maceration and the resulting tissue extract mixed thoroughly. The supernatant fluid was used for all subsequent bacteriological examination (Ayres, in the Press).

Enumeration of E. coli *and coliforms*

One ml. quantities of the tissue suspension were inoculated into each of 20 roll tubes containing 4 ml. of MacConkey agar (Oxoid No. 3 roll tube) according to the method of Reynolds & Wood (1956); ten tubes were incubated at 37° C. and ten at 44 ± 0·2° C. for 24 hr. Counts of red, lactose-fermenting colonies at each temperature were taken as estimates of the numbers of coliforms and *E. coli* respectively.

Enumeration of faecal streptococci

These bacteria were estimated by a most probable number (MPN) technique using thallous acetate broth (Barnes, 1956) incubated at 37° C., with subculture to 0·04% potassium tellurite plates. Additional confirmation of representative small, discrete, black colonies was provided by Gram-staining.

Enumeration of Clostridium welchii

A MPN technique was also used for estimating *Cl. welchii*; primary culture of the extract and its dilutions into cooked meat broth incubated at 37° C. for 48 hr. was followed by subculture to a modified Nagler medium (Willis & Hobbs 1959). Inhibition of Nagler reaction by specific *Cl. welchii* antitoxin was taken as a positive result.

Enumeration of total plate count (TPC)

Total plate count estimates were made from decimal dilutions of the sample (prepared in 0·1% peptone water), using the drop count technique of Miles & Misra (1938). Two 0·02 ml. drops of each dilution were placed on replicate plates of blood agar and nutrient agar for incubation at 20, 30 and 37° C. Colony counts were made with a low-power binocular microscope at daily intervals until no further increases were observed; generally this took 24 hr. at 37° C., 48 hr. at 30° C. and 72 hr. at 20° C.

Computation of bacterial count of shellfish

The mean numbers of *E. coli* and coliforms/ml. of tissue were estimated by doubling the mean number of colonies present in each set of ten tubes inoculated. Estimates of faecal streptococci and *Cl. welchii* were computed from MPN tables (Report, 1970). Where possible, the total plate count was computed from the dilution yielding between 20 and 100 colonies/0·02 ml. drop.

RESULTS

A total of 169 samples were examined by the methods described. Although this sampling did not include some minor sources of shellfish production the frequency of sampling from any one source was roughly related to total commercial production from that source (see Table 1).

Table 2. *Percentage distribution of* Escherichia coli *and coliforms in market shellfish*

E. coli (166 samples)		Coliforms (161 samples)	
Colonies/ml. of tissue	% of samples	Colonies/ml. of tissue	% of samples
0–2	92·8	0–5	59·1
2–5	2·3	5–10	8·1
5–10	1·25	10–25	4·9
10–15	0·75	25–50	4·5
>15	2·95	50–100	7·9
		>100	15·5

Table 3. *Percentage distribution of faecal streptococci and* Clostridium welchii *in market shellfish*

Faecal streptococci (166 samples)		*Clostridium welchii* (167 samples)	
MPN/ml. of tissue	% of samples	MPN/ml. of tissue	% of samples
0–10	60·5	0–2	78·0
10–50	8·6	2–5	10·25
50–100	0·7	5–10	1·2
100–500	4·6	10–50	9·1
>500	25·7	>50	1·45

E. coli *and coliform numbers*

Table 2 shows the distribution of these organisms in the samples, expressed as a percentage of the total number of samples examined. A high proportion (95·1 %) of the samples fell within the generally accepted standard of five *E. coli*/ml. tissue (Sherwood & Thomson, 1953) used by market and public health authorities in the United Kingdom. The majority of the samples had been treated in a purification plant before marketing and, using the more rigid standard employed for shellfish from this source, i.e. not exceeding 2/ml. (Wood, 1963), 92·8 % of the samples were acceptable by this standard. The *E. coli* estimates were consistent with the origins of the samples in that those with higher counts were taken from areas known (from field surveys) to be polluted by sewage.

As expected, coliform counts were higher and subject to greater variation than *E. coli* counts, but because of their less specific association with human faecal pollution the significance of coliform numbers was not always clear. The highest counts were found in mussel and oyster samples and were more often associated with unpurified samples from areas known to be polluted by sewage. The numbers of coliforms were possibly influenced by the handling conditions of samples before receipt, since these organisms can increase in stored shellfish, particularly at temperatures above 10° C. (Hoff & Presnell, 1963; Wood, 1964).

Table 4. *The coliform, faecal streptococci and* Clostridium welchii *content of shellfish in relation to numbers of* Escherichia coli

	Coliforms			Faecal streptococci			*Clostridium welchii*		
E. coli ml. of tissue	No. of samples*	Range/ml.	Mean/ml.	No. of samples*	Range/ml.	Mean/ml.	No. of samples*	Range/ml.	Mean/ml.
0–2	150	0–1600	99·8	154	0–>1800	>548·7	154	0–113	2·6
2–5	3	5·6–>200	>135·2	3	0·2–>1800	>900	3	0–4	1·9
5–10	3	3–>200	>69·3	2	5–>1800	>902·5	2	1·4–25	13·2
10–15	0	—	—	0	—	—	0	—	—
>15	3	79·8–150	121·3	4	0·7–180	138·9	4	1·2–160	50·6

* Number of samples where counts were made of *Escherichia coli* and of organism shown.

Table 5. *Percentage distribution of total plate counts (TPC) of market shellfish at incubation temperatures of* 20, 30 *and* 37° *C.*

Colony count/ ml. of tissue	Nutrient agar			Blood agar		
	20° C.	30° C.	37° C.	20° C.	30° C.	37° C.
$< 10^5$	24·8	21·1	24·35	25·8	24·8	28·6
$10^5 < 10^6$	27·6	27·6	33·25	28·3	25·8	27·3
$10^6 < 10^7$	41·9	45·9	38·7	41·4	43·7	39·15
$\geqslant 10^7$	5·7	5·4	3·7	4·5	5·7	4·95

Faecal streptococci

Table 3 gives the distribution of samples containing these organisms, expressed as a percentage of the total number of samples examined. No apparent relation could be demonstrated between the numbers of *E. coli* or coliforms and those of faecal streptococci but there was evidence of seasonal differences affecting the numbers of faecal streptococci in the samples. Counts in samples taken between September and December were higher than in the samples taken between January and March, when low water temperatures and reduced feeding activity probably affected the degree of bacterial concentration by the shellfish.

Clostridium welchii

The distribution of numbers of samples containing these organisms, expressed as a percentage of the total number examined, is shown in Table 3. The majority of samples contained less than 2/ml. of tissue, and those containing more than 10/ml. came from sources yielding high coliform counts. The samples containing more than 50/ml. also contained the highest number of *E. coli*; these samples were of mussels destined for heat processing.

There appeared to be a direct relation between the numbers of *E. coli* and *Cl. welchii* but not between *E. coli* and coliforms or faecal streptococci (Table 4).

Total plate counts (TPC)

Because of the wide range of estimates obtained from the combination of media and incubation temperatures, the TPC data have been summarized in Table 5 by broadly grouping the observations. No distinction has been drawn between results from the four species of shellfish examined.

The results indicate that estimates were not influenced by the medium or temperature of incubation. However, there was considerable variation between individual samples: TPCs ranged from 10^3 to 10^8/ml. but there was no evidence of any seasonal trend, possibly because of changes in the bacterial flora which took place during storage and marketing. In conducting storage experiments with live shellfish, Wood (1964) noted that TPCs in the range 0·5 to 1×10^6 bacteria/ml. of oyster tissue appeared to be the maximum consistent with storage under good conditions of time and temperature. From the current data, between 42 and 51 % of the samples examined yielded results in excess of 10^6 bacteria/ml.

Between 3·7 and 5·7 % of the samples examined contained in excess of 10^7

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Table 6. *The relation between* 20 *and* 37° *C. total plate counts of shellfish sampled from different sources*

Source	No. of samples	Mean count on blood agar		Ratio 37°/20° count
		20° C.	37° C.	
East coast				
A	18	$2{\cdot}7 \times 10^6$	3×10^6	1·11
B	14	$3{\cdot}9 \times 10^6$	$3{\cdot}5 \times 10^6$	0·9
C	13	$1{\cdot}8 \times 10^6$	$1{\cdot}7 \times 10^6$	0·94
South coast	13	$1{\cdot}1 \times 10^6$	$7{\cdot}3 \times 10^5$	0·66
South-west coast				
A	11	$3{\cdot}3 \times 10^6$	$6{\cdot}3 \times 10^6$	1·91
B	21	$3{\cdot}4 \times 10^6$	$3{\cdot}9 \times 10^6$	1·15
C	11	2×10^6	$1{\cdot}3 \times 10^6$	0·65
D	19	$3{\cdot}4 \times 10^7$	$2{\cdot}8 \times 10^7$	0·82
E	4	$8{\cdot}8 \times 10^6$	$7{\cdot}3 \times 10^6$	0·83
F	7	$3{\cdot}2 \times 10^6$	$2{\cdot}1 \times 10^6$	0·66
Coast of Wales				
A	9	$1{\cdot}6 \times 10^6$	$1{\cdot}0 \times 10^6$	0·62
B	4	$6{\cdot}5 \times 10^7$	$8{\cdot}8 \times 10^7$	1·35
Coast of Ireland	14	$4{\cdot}6 \times 10^6$	2×10^6	0·45

bacteria/ml.; these were either oysters from two of the sources which yielded more than 5 *E. coli*/ml. or mussels which were in a weak condition, having been out of water for up to 3 days before final sale.

Analysis of the total plate counts of shellfish according to their origin (Table 6) showed that shellfish from the majority of sources contained more non-specific bacteria growing at 20° C. than at 37° C. The shellfish from four sources yielded more non-specific bacteria at 37° C. than at 20° C.

DISCUSSION

The majority of samples taken in this investigation were found, by current British standards, to be bacteriologically suitable for consumption, containing less than 5 *E. coli*/ml. tissue. In general, the numbers of faecal streptococci, coliforms and *Cl. welchii* were so low that they were considered unlikely to pose any direct public health problem. The numbers of faecal streptococci, coliforms, *Cl. welchii* and of non-specific bacteria in many samples could not be correlated with the 'pollution' characteristics of the area of origin, with *E. coli*, or with each other, and it would be difficult to define any base-lines for acceptance or rejection of shellfish using these criteria.

The significance of the total plate counts is open to a number of interpretations, since there are no readily comparable base-lines. Those shellfish containing more than 10^7 bacteria/ml. were derived from two sources (south west source D, Welsh coast source B), one of which was associated with sporadic incidents of oyster-borne non-specific illness (south west source D); further investigation is required to

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establish whether any causal relationship exists. In the present investigation, shellfish from these sources were often found to contain evidence of sewage pollution, and the TPCs made at 37° C. were particularly high. It has been suggested that food poisoning is often associated with large numbers of non-specific bacteria growing at 37° C. and that numbers in excess of 10^7/g. are significant (Ingram, 1961).

Despite the occurrence in mussels of large numbers of bacteria capable of growing at 37° C., in the United Kingdom this species has not been associated with illness of the non-specific type, perhaps because mussels are usually eaten cooked. The results obtained here suggest that oysters from sources which have been associated sporadically with illness contain more non-specific bacteria capable of growing at 37° C. than at 20° C., in contrast to oysters from the majority of sources which yielded larger numbers of bacteria growing at 20°C. than at 37° C.

The 42–51 % of samples with more than 10^6 bacteria/ml., which Wood (1964) proposed might be consistent with the maximum for oysters stored under good conditions, emphasizes the need for qualitative as well as quantitative data. The public health significance of such figures is difficult to interpret since no standards exist and there is a lack of epidemiological evidence of the importance of these organisms in shellfish. Hunter & Linden (1923) working with shucked shellfish (i.e. removed from the shell) found no apparent correlation between the total plate count and the stage of decomposition, and it was suggested that bacterial types rather than absolute numbers were an important factor affecting decomposition. In the present study the majority of shellfish samples which contained more than 10^7 organisms/ml. were considered acceptable to the consumer, as judged by the *E. coli* test and by visual examination. However, earlier work undertaken in this laboratory showed that total plate counts of bacteria in oysters from an acceptable single source ranged over the year from 10^3 to 10^7/g. and were directly related to seasonal water temperatures; the numbers of bacteria appeared to be related to the feeding activity of the shellfish. Unpublished work has been undertaken by Miss Sheila Halls at this laboratory to determine bacterial changes taking place during the purification of oysters in experimental tanks under a wide range of controlled conditions (water temperature, water flow, density, etc.). It has been found that although a system may be effective in removing bacteria of faecal origin (e.g. *E. coli*) the total bacterial count is not significantly affected by variations in the conditions of purification. Thus, there is considerable evidence that substantial numbers of non-specific bacteria are always present in oysters, even after they have ejected material from their alimentary tract under controlled conditions of purification.

These studies highlight an important difference between the bacterial flora of molluscan shellfish and that of related foods such as wet fish; there is a vast difference between the numbers of bacteria present in shellfish and wet fish when taken from the water. The surface viable count of wet fish is usually below 10^3/g. (Shewan, 1970) but may subsequently increase as the result of handling and processing. The results reported here confirm that the numbers of bacteria in live raw molluscan shellfish are generally much in excess of those for wet fish.

The identification of the bacterial flora of market shellfish has proved difficult because of the large number of strains which must be examined and the need for many tests to place the bacteria into even broad taxonomic groups. Even then, the possible significance or public health role of bacteria not generally recognized as pathogenic cannot be readily interpreted from existing knowledge.

As many shellfish, particularly oysters, are consumed as live raw products a microbial standard for non-specific bacteria might seem desirable, but until epidemiological evidence supports more precise definition it is impracticable to define such a standard, since relatively large numbers of bacteria are found in freshly caught shellfish even from clean areas. At the present time any standard would be purely arbitrary. In the formulation of such a standard, however, recognizing the inherent variability of bacterial counts, it would seem prudent, as Ingram (1961) suggested, to express the standard in such a way as to limit the percentage of samples exceeding an agreed value. The ratio of the numbers of non-specific bacteria in tissue at 20 and 37° C. and its relation to the incidence of non-specific illness following the consumption of raw shellfish might prove a useful tool in the study of the epidemiology of incidents related to shellfish.

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