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Food poisoning caused by heat-sensitive *Clostridium welchii*. A report of five recent outbreaks

By R. G. A. SUTTON and BETTY C. HOBBS
Food Hygiene Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W. 9

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INTRODUCTION

The earliest reports linking *Clostridium welchii* with food poisoning were by Klein (1895) and Andrews (1899). In both instances the patients suffered mild diarrhoea but no vomiting. The investigations made in both these outbreaks were, however, not sufficient to establish with certainty the causative role of the organism.

McClung (1945) in the U.S.A. reported four outbreaks of food poisoning from chicken dishes. The symptoms, appearing 8–12 hr. after the patients had eaten the meal, were intestinal cramp and diarrhoea with little vomiting. Examination of the remains of the chickens revealed the presence of large numbers of *Cl. welchii*. Hobbs *et al.* (1953) published the results of the epidemiological and bacteriological studies of several outbreaks of food poisoning occurring in the U.K. during the period September 1949 to February 1952. In all these incidents the causative organism was a strain of *Cl. welchii* that was non-haemolytic on horse blood agar, produced only trace amounts of α-toxin and formed spores capable of withstanding 100° C. for periods of 1–4 hr. Since then this heat-resistant variant of *Cl. welchii* has come to be regarded as a major cause of food poisoning in the U.K. (Hobbs, 1965), and reports from other countries (Sutton, 1966; Dauer, 1961, Hayashi, Kugita, Tawara & Yamagata, 1961) have increasingly incriminated heat-resistant *Cl. welchii* as a cause of food poisoning.

Heat-sensitive strains of *Cl. welchii* have been reported as the cause of food poisoning on only a few occasions. McKillop (1959) described an outbreak in which a β-haemolytic, heat-sensitive strain was thought to be the causative organism; while Taylor & Coetzee (1966) reported an outbreak due to non-haemolytic, heat-sensitive *Cl. welchii*. In the U.S.A. Hall, Angelotti, Lewis & Foter (1963), after examining the characteristics of many *Cl. welchii* isolated from contaminated foods and food associated with food poisoning outbreaks, concluded that heat-sensitive strains of *Cl. welchii* had been responsible for food poisoning outbreaks in that country.

This paper describes five outbreaks of food poisoning due to heat-sensitive *Cl. welchii* investigated at the Food Hygiene Laboratory, London, in conjunction with regional Public Health Laboratories in England, between September 1966 and May 1967, together with four outbreaks in which both heat-sensitive and heat-resistant strains are thought to have been involved.

MATERIALS AND METHODS

Media used

Neomycin blood agar was prepared by allowing 5–10 ml. of 1% peptone water agar to set in a Petri dish and covering it with a layer of 10 ml. 5% horse blood agar. When set 3–5 drops (0·06–0·1 ml.) of 1% neomycin sulphate were spread evenly over the surface with the aid of a bent glass spreader. The plates were then dried before use. This medium inhibits the growth of coliform bacilli and most other organisms occurring in the faeces and allows the ready isolation of *Cl. welchii*. It is important to incubate for only 24 hr., as prolonged incubation permits other organisms to grow.

Egg yolk medium was prepared by the method of Willis & Hobbs (1958); one half of the plate was spread with 3 drops of *Cl. welchii* type A antitoxin (Burroughs Wellcome) immediately before use in order to demonstrate the inhibition of the lecithinase reaction.

Cooked meat medium (C.M.M.) made with veal was used throughout.

Sporulation medium. The sporulation medium was made according to Roberts (1968) with added glucose and thioglycollate (TPAY–CT).

Serology

Hobbs type antisera 1–17 of the Food Hygiene Laboratory collection were used. Where necessary a new antiserum was prepared (see below).

Examination of faeces

All faecal samples were examined using the following three techniques; (1) Direct viable cell count on an unheated faecal suspension. (2) Spore count on a faecal suspension heated at 80° C. for 10 min. and (3) Enrichment culture following heating in C.M.M. for 60 min. at 100° C.

Viable cell and spore counts of Cl. welchii

A 1/100 suspension of faeces was made by emulsifying 1 g. of faeces in 1–3 ml. of quarter strength Ringer's solution; the volume was made up to 100 ml. in a 4 oz. bottle. With this suspension serial tenfold dilutions were made to give a final dilution range of 10^2 to 10^5. Viable cell counts were carried out on neomycin blood agar using the technique of Miles & Misra (1938).

The 1/100 faecal suspension was heated at 80° C. for 10 min., and the viable count repeated. In this way the number of viable spores present could be estimated.

Isolation of heat-resistant Cl. welchii

A small portion (approximately 1 g.) of faeces was emulsified in each of two 1 oz. bottles of cooked meat medium and immediately placed in a water bath at 60–70° C. The water was boiled and one bottle was removed after 30 min., and the other after 60 min. at 100° C. The bottles were incubated at 37° C. overnight and subcultured the following day on neomycin blood agar.

All plates were incubated anaerobically at 37° C.

Identification of Cl. welchii

Colonies showing the typical appearance of *Cl. welchii* (Cruickshank, 1965) were further examined by Gram stain, lactose fermentation and inhibition of the lecithinase reaction on egg yolk medium by *Cl. welchii* type A antiserum.

The organisms were serotyped using Hobbs's sera 1–17, by the method of Hobbs *et al.* (1953). When the strains could not be typed in this way, an antiserum was prepared against the suspected organism, preferably selected from food, by the method of Henderson (1940).

Determination of heat resistance of Cl. welchii *spores*

The heat resistance of the organisms responsible for outbreaks 1 to 5 was further investigated. Spore suspensions were prepared by the method of Roberts (1968). The yield of spores was not as good as that obtained by Roberts, but was, nevertheless, satisfactory for semi-quantitative studies of heat resistance.

One ml. amounts of the washed spore suspension were inoculated into nine 1 oz. bottles of C.M.M., which were held at 100° C., three for 10 min., three for 20 min. and three for 30 min. All were removed, cooled and incubated at 37° C. for 48 hr. They were then subcultured on blood agar medium and incubated anaerobically at 37° C. for 24 hr. Results were recorded as presence or absence of *Cl. welchii*.

RESULTS

The heat resistance of an organism is generally expressed in one of two ways.

1. *Quantitatively.* By the use of such terms as D and Z values it is possible to express quantitative data on the heat resistance of an organism over a wide temperature range. *D* values are usually determined by suspending the organisms in buffer solution in sealed ampoules, heating at various temperatures and doing viable cell counts in order to ascertain the number of survivors after a known heat treatment. Heat-resistant spores of *Cl. welchii* have a D_{100} of 5 min. or longer (Roberts, 1968). The technique, although the most accurate, is somewhat laborious and is often replaced by the following technique.

2. *Semi-quantitatively.* The semi-quantitative results of heat resistance studies are usually expressed as 'Thermal death times'. They are determined by inoculating the test organism (often in unknown numbers) into tubes of medium, heating for varying periods of time and testing for the presence or absence of survivors. In the case of 'typical food poisoning strains', it has been well established that even small numbers of organisms will survive 100° C. for at least 1 hr. when heated in C.M.M. (Hobbs *et al.* 1953; Collee, Knowlden & Hobbs, 1961); whereas even a large inoculum of classical β-haemolytic *Cl. welchii* will not yield survivors after 15 min. at 100° C. when heated in C.M.M.

As this paper deals with the isolation of *Cl. welchii* from faeces, the term heat-resistant will imply that the organism could be isolated from a faecal suspension after heating in C.M.M. for 1 hr. at 100° C. Heat-sensitive will imply that the organism could not be isolated from faeces in this way. More detailed information on the heat resistance of the causative organisms is given in Table 2.

Outbreak 1

The incident occurred in October 1966 at a school in the Liverpool area.

On the morning of 18 October, 77 lb. of meat were cut into fourteen pieces of approximately equal size and cooked for 3–4 hr. in a steamer in 3 cylindrical pans approximately 18 in. in diameter and 12–15 in. deep. They were then allowed to cool from 12.30 p.m. to 9.30 p.m. The pieces were removed from the pans and refrigerated until about 10.45 a.m. on the 19th, when they were sliced and eaten cold.

Of 400 persons at risk 50 developed abdominal pain and diarrhoea within 12–15 hr. No vomiting occurred. The illness lasted 12–24 hr.

Bacteriology

The incriminated meat and faeces from seventeen persons affected were examined for *Cl. welchii*. Beta haemolytic *Cl. welchii* were found in the food, and in large numbers ($7{\cdot}5 \times 10^5 - 2{\cdot}5 \times 10^7$/g.) in fifteen of the seventeen specimens of faeces. Beta haemolytic strains were isolated after 30 min. but not 60 min. boiling from six specimens of faeces, but on re-examination of the faeces only 1 strain survived 30 min. boiling. In no case were non-haemolytic, heat-resistant strains isolated after 30 or 60 min. at 100° C. None of the strains isolated was typable by Hobbs type 1–17 antisera. An antiserum was therefore prepared against the strain isolated from the meat and it agglutinated 14/15 β-haemolytic strains isolated from the faeces. Toxicologically this strain behaved as a typical type A strain, producing α, κ and θ toxins.

Outbreak 2

This outbreak occurred in the London area in October 1966 following a harvest supper. Two frozen turkeys, each approximately 21 lb. in weight, were delivered to the premises on Friday evening. They were thawed for 36 hr. in the polythene bags before being stuffed; the stuffing contained sausage meat.

They were cooked on Sunday afternoon for 6 hr.—1 hr. at 195° C. followed by 3 hr. at a lower temperature and finally 2 hr. at 195° C. They were then removed and allowed to cool on top of the oven for 2 hr. before they were put back into the 'warm' oven for storage overnight. The next day the meat was sliced, piled on an unknown number of plates and rewarmed in the oven before serving.

Symptoms of diarrhoea and abdominal pain occurred 8–12 hr. after the ingestion of the food, in 27 of 120 persons who ate the meal. One patient, a 62-year-old woman, died 48 hr. after eating the meal. She was suffering from Pott's disease at the time. The cause of death was certified as 'acute enterocolitis due to food poisoning'.

Bacteriology

Unfortunately no food was available for examination and faeces were not collected until 7–9 days after the onset of the illness. Faeces from twenty-one patients were examined, and of these, twenty contained β-haemolytic and fourteen non-haemolytic *Cl. welchii*, generally in relatively small numbers on direct culture.

In no case were heat-resistant *Cl. welchii* isolated. Only two strains, both β-haemolytic, were typable. Antisera were, therefore, prepared against three of the non-haemolytic strains but no serological relationship between the fourteen strains isolated could be established. On the other hand an antiserum prepared against a β-haemolytic strain agglutinated 11/19 β-haemolytic strains tested.

As the faeces were not collected until 7–9 days after the onset of symptoms it must be expected that the direct counts would have dropped to within the limits obtained. These findings therefore strongly suggest that the outbreak was caused by a β-haemolytic, heat-sensitive strain of *Cl. welchii*.

Outbreak 3

The outbreak followed a meal, eaten by four persons, in a Chinese restaurant. Common items of food were grilled ox liver and ice cream. Symptoms of abdominal pain and diarrhoea occurred in all four persons 10–14 hr. after eating the meal.

Deep frozen South American ox liver was received at the Chinese restaurant 24 hr. before it was cooked. It was kept frozen for 12 hr. before it was transferred to a 4° C. refrigerator, and grilled 1–2 hr. before the meal.

Bacteriology

Faeces were collected from all four persons 24–48 hr. after the symptoms occurred. All four faeces contained large numbers of non-haemolytic *Cl. welchii* ($1{\cdot}7 \times 10^7 - 5{\cdot}0 \times 10^7$/g.) but *Cl. welchii* could not be isolated from the faeces after 30 min. at 100° C. Serologically all four strains were Hobbs type 5.

Unfortunately none of the incriminated liver was left, but the examination of similar samples yielded large numbers of haemolytic *Cl. welchii* (not typable), indicating that the liver was a potential source of *Cl. welchii*.

Outbreak 4

The outbreak occurred in April 1966 in a school canteen. On 5 April 200 children ate a mid-day meal of cold, rolled roast beef, vegetables, jam tart and custard. The beef was cooked the previous day—how long it had been cooked, and at what temperature, could not be ascertained, but samples of meat examined appeared to be adequately cooked (in a culinary sense); it was said to have been refrigerated immediately after cooking, sliced the following morning and immediately refrigerated until lunch time, when it was served.

Symptoms of diarrhoea and abdominal pain occurred in thirty of the 200 persons 10–11 hr. after the meal. There was no vomiting and the illness was of brief duration.

Bacteriology

A direct Gram stain of the beef showed fairly large numbers of organisms, mainly Gram-positive bacilli and some cocci. A moderate growth of both haemolytic and non-haemolytic *Cl. welchii* was obtained from the beef.

Six samples of faeces were collected from persons with symptoms. In all samples haemolytic *Cl. welchii* were present in large numbers ($6{\cdot}0 \times 10^6 - 1{\cdot}0 \times 10^8$/g.) in

direct culture. In no case were *Cl. welchii* grown after heating faeces at 100° C. for 30 min.

Serologically the organism was not agglutinated by Hobbs types 1–17 antisera, and an antiserum was prepared against the β-haemolytic strain isolated from the beef. This antiserum agglutinated all the six β-haemolytic strains isolated from the faeces.

Table 1. *Viable cell counts of heat sensitive* Cl. welchii *in faeces of persons associated with food poisoning outbreaks 1–5*

Outbreak no.	No. faeces examined (1)	No. containing causative org. (2)	Median v.c.c. of *Cl. welchii* Total (T) (3)	Median v.c.c. of *Cl. welchii* Spores (S) (4)	Ratio S:T (5)*
1	17	14	$4{\cdot}0 \times 10^6$	$4{\cdot}0 \times 10^5$	1:10
2	21	11	$1{\cdot}0 \times 10^3$	$2{\cdot}5 \times 10^2$	1:5
3	4	4	$5{\cdot}0 \times 10^7$	$1{\cdot}0 \times 10^7$	1:5
4	6	6	$3{\cdot}5 \times 10^7$	$1{\cdot}5 \times 10^7$	1:3
5	11	11	$9{\cdot}5 \times 10^6$	$2{\cdot}0 \times 10^6$	2:3

* The values in this column do not indicate value of column 4/column 3. They indicate the median value of the individual S:T ratios calculated for each faeces of the outbreak involved.

Table 2. *Results of the heat resistance tests on spore suspensions grown in laboratory medium of the strains of* Cl. welchii *responsible for the food poisoning outbreaks 1–5*

Outbreak no.	Initial spore count	Number of tubes showing survivors after heating at 100 °C. for 10 min.	20 min.	30 min.
1	$1{\cdot}5 \times 10^4$	1/3	0/3	0/3
2	$3{\cdot}5 \times 10^3$	0/3	0/3	0/3
3	$5{\cdot}0 \times 10^4$	1/3	0/3	0/3
4	$1{\cdot}0 \times 10^3$	0/3	0/3	0/3
5	$4{\cdot}5 \times 10^3$	1/3	0/3	0/3

Outbreak 5

An outbreak occurred in May 1967 in a school canteen in the Salisbury area. On 1 May three uncooked 'frozen' tongues were delivered to the canteen, but one of the staff noted that the tongues were not truly frozen. They were cooked in a steamer for 4 hr., each in a different container, removed, skinned and pressed. They were said to have been refrigerated within $\frac{1}{2}$ hr. of removal from the steamer. At 9.15 a.m. the following day they were removed from the refrigerator, sliced and prepared for the mid-day meal, which was eaten cold by 206 children and nineteen members of the staff.

Eighty-eight persons suffered symptoms of abdominal pain and diarrhoea, which began 9–10 hr. after the meal and continued into the early hours of the next morning. Recovery was fairly rapid that day.

Bacteriology

The remains of the tongue were cultured and found to contain 10^7 β-haemolytic *Cl. welchii* per gram. Faeces were collected from eleven persons 48 hr. after onset of symptoms and β-haemolytic *Cl. welchii* were isolated by direct culture in large numbers ($2{\cdot}0 \times 10^5 - 3{\cdot}0 \times 10^7$/g.) from all specimens. No *Cl. welchii* were isolated after heating at 100° C. for 30 min. All strains isolated from food and faeces gave a weak agglutination with Hobbs type 5 antiserum but as the reaction was only weak an antiserum was prepared against the strain isolated from the tongue. This antiserum agglutinated all the eleven strains isolated from the faeces.

The results from outbreaks 1–5 together with the results of the studies of heat resistance are summarized in Tables 1 and 2.

Outbreaks due to both heat-resistant and heat-sensitive Cl. welchii

In addition to the five outbreaks described, four incidents have occurred in which both heat-resistant and heat-sensitive *Cl. welchii* have been considered significant. A brief outline of each is given below.

(i) Heat-sensitive, non-haemolytic *Cl. welchii* type 7 was isolated by direct culture in large numbers (*c.* 10^7 orgs/g.) from 7/7 persons who had symptoms of diarrhoea and abdominal pain after eating boiled beef and mince. Heat-resistant, non-haemolytic *Cl. welchii*, all type 4, were isolated from 4/7 faeces, but presumably only in small numbers as they could not be isolated by direct culture after heating at 100° C. for 30 min. in cooked meat. This can be readily accomplished if the heat-resistant strains are present in numbers of 10^4/g. or greater. Unfortunately none of the incriminated meat was available for examination.

(ii) This outbreak occurred following a mid-day meal at a teachers' training college. Symptoms of diarrhoea and abdominal pain occurred in 123 of 320 persons at risk 8–24 hr. after eating the meal of roast pork. Heat-sensitive, non-haemolytic *Cl. welchii*, not type 1–17 were isolated directly in large numbers from 14/15 specimens of faeces. An antiserum prepared against one strain agglutinated 11/14 strains tested. In addition all fifteen faeces contained large numbers of heat-resistant non-haemolytic *Cl. welchii* type 3. No pork was available for examination.

(iii) In this outbreak symptoms of diarrhoea and abdominal pain occurred in 60 of 180 geriatric hospital patients 10 hr. after they had eaten a meal of minced meat. Heat-sensitive and heat-resistant strains of *Cl. welchii* (both non-haemolytic) were isolated in large numbers (*c.* 10^6/g.) from the beef. Antisera were prepared against both strains. The antiserum against the heat-resistant strain agglutinated a non-haemolytic, heat-resistant strain isolated in large numbers from 9/9 faeces. Similarly the antiserum against the heat-sensitive strain agglutinated 8/9 heat-sensitive strains isolated from the faeces.

(iv) This outbreak occurred in a hospital and followed a meal of cold roast pork. Twenty seven of 300 persons who ate the pork suffered symptoms of mild diarrhoea 8–12 hr. after the meal. Eleven samples of faeces were collected, and both heat-resistant, non-haemolytic *Cl. welchii* type 13, and heat-sensitive, non-

haemolytic *Cl. welchii* Type 1 were isolated in large numbers from all eleven faeces. No *Cl. welchii* could be isolated from the sample of meat examined.

DISCUSSION

Hauschild, Niilo & Dorward (1967) and Hauschild & Thatcher (1967), in feeding experiments carried out with human volunteers and lambs, used heat-sensitive non-haemolytic *Cl. welchii* and were able to produce symptoms of food poisoning similar to those associated with heat-resistant *Cl. welchii*. This, with earlier reports of McKillop (1959) and Taylor & Coetzee (1966), and the results described in the present paper, demonstrates that heat-sensitive strains of *Cl. welchii* are capable of causing food poisoning in man.

Heat-sensitive *Cl. welchii* are present in nature far more frequently than the heat-resistant strains; it is therefore pertinent to ask why food poisoning due to heat-sensitive *Cl. welchii* has not been reported more frequently in the past. There are two probable explanations; the natural variation in the heat resistance of the spores which enables the heat-resistant strains to survive the cooking process far more frequently, and the difficulty of isolating the heat-sensitive strains by the techniques used in the past.

It has been shown (Hobbs, 1965) that *Cl. welchii* food poisoning is due to the survival during cooking of the organism already present in the raw meat followed by germination of spores and multiplication of the organism in the non-refrigerated meat dish. The heat-resistant nature of the spore had always been considered an integral part of this process. For this reason heat-sensitive strains of *Cl. welchii* have only occasionally been considered as a cause of natural outbreaks of food poisoning. The work of Hauschild & Thatcher (1967) clearly demonstrated that the organism could cause food poisoning, but it did not demonstrate in any way that the organism could survive the cooking process and cause food poisoning under conditions similar to those occurring in a natural outbreak.

Sylvester & Green (1961) investigated the temperature gradient within and on the surface of meat samples during slow and conventional roasting. They demonstrated that the usual conventional method of roasting was bacteriologically safer than slow overnight roasting. In the conventional method the temperature within the meat reached 85–90° C., while in the overnight roasting the temperature reached only 65° C.

More recently Woodburn & Kim (1966) have shown that heat-sensitive *Cl. welchii* inoculated into the stuffing of turkey could survive a normal roasting procedure, in fact, the temperature in the deep tissue of the turkey never rose above 82° C. They were readily able to detect survivors from turkey stuffing subjected to a long slow roast (oven temperature 94° C. for $16\frac{1}{2}$ hr.) and a short fast roast (oven temperature of 232° C. for $2\frac{3}{4}$ hr.). They concluded that in meats the temperature at the end of cooking for a well-done product is about 85° C.

In outbreak 2 the temperature of the inner turkey meat probably never rose above 75–80° C. and must have been at this temperature for only a short time. The size of the turkey/time of cooking ratio leads one to this conclusion.

Although the 'classical' strains of *Cl. welchii* are considered to be heat-sensitive in contrast to the marked heat resistance of the 'typical food poisoning' strains, it must still be remembered that the spores of the heat-sensitive strains are much more heat-resistant than normal vegetative cells and a limited number of quantitative studies in this laboratory (unpublished) have shown that in meat medium they can withstand a temperature of 80° C. for relatively long periods of time (D 80 meat = 60–200 min.).

It is therefore reasonable to conclude that heat-sensitive *Cl. welchii* may, under favourable conditions, survive a cooking process, particularly if a large piece of meat is being cooked for a relatively short period of time, as occurred in outbreak 2. This likelihood is increased if the meat was previously frozen, as the three outbreaks due to frozen meats reported here demonstrate. The finding that three of the five outbreaks described in detail were due to frozen meats is particularly interesting. The duration and method of thawing have a critical effect on the temperature that might be reached within the meat during cooking, and further investigations should pay attention to these points.

The role of contamination after cooking is not clear. McKillop (1959) clearly indicates that the dust in the air and in vessels used for storage may play a prominent part in *Cl. welchii* food poisoning, although much of the epidemiological evidence to date (admittedly concerned primarily with heat-resistant *Cl. welchii*) assumes that the causative strain of *Cl. welchii* is almost always present in the meat before cooking. Much work is still to be done to clarify this point, particularly in regard to β-haemolytic *Cl. welchii*. It should be remembered that, in contrast to the non-haemolytic heat-resistant strains, the spores of classical strains germinate fairly readily without prior heat treatment (Roberts, 1968) and that both heat-sensitive and heat-resistant strains require anaerobic conditions for growth.

Until recently most workers in the U.K. may have considered *Cl. welchii* food poisoning to be caused only by the heat-resistant non-haemolytic strain of *Cl. welchii* type A. So much so, in fact, that the term 'typical food poisoning' as apart from 'classical' *Cl. welchii* has been developed to describe this variant. As a result a standardized technique of heating the suspected faeces in C.M.M. for 60 min. at 100° C. before overnight incubation has been adopted in examining faeces from suspected outbreaks of *Cl. welchii* food poisoning. Direct culture of faeces on neomycin blood agar incubated anaerobically has not been routinely carried out. This technique therefore precludes the possibility of incriminating heat-sensitive *Cl. welchii* as the cause of food poisoning outbreaks.

It therefore appears likely that heat-sensitive *Cl. welchii* is the cause of food poisoning in far more instances than present reports indicate. It may in fact be responsible for some of the incidents of food poisoning in the past for which no cause has been found. Future reports of food poisoning should pay particular attention to finding out whether the method of examination used was satisfactory for the detection of heat-sensitive *Cl. welchii*. Only in this way can a true indication of the ratio of outbreaks due to heat-sensitive and heat-resistant strains be obtained, and the significance of this heat-sensitive strain of *Cl. welchii* as a cause of food poisoning be assessed.

Sutton (1966) investigated one outbreak of *Cl. welchii* food poisoning and reported large numbers of the causative organism in the faeces of persons involved. This finding has been confirmed in this work, and also in investigations of many outbreaks due to heat-resistant *Cl. welchii* (unpublished), provided the specimens of faeces are collected within 3 days of the onset of symptoms. The viable cell count by the Miles and Misra method is generally of the order of *c.* 10^5–*c.* 10^7 *Cl. welchii*/gram; there is tendency for the count to decrease with time, so that specimens collected 1 week after symptoms have occurred may be within normal limits (median count approximately 10^3 *Cl. welchii*/gram). The increase in count may be useful in investigating outbreaks of food poisoning due to heat-sensitive *Cl. welchii*. As haemolytic, heat-sensitive *Cl. welchii* occur (mostly in small numbers) in almost all faeces (Collee *et al.* 1961), some idea of the numbers present is of importance. The finding of large numbers of *Cl. welchii* in a group of suspected faeces would certainly warrant full serological investigation, with the possible preparation of an antiserum. In some of the outbreaks investigated (due to either heat-sensitive or heat-resistant *Cl. welchii*) it has been noted that as well as multiplication of the causative organism, multiplication of strains of *Cl. welchii* already present in the intestine has occurred. Freshly collected faecal samples may therefore contain large numbers of the causative strain along with other strains of *Cl. welchii*. Thus there may be difficulties in the interpretation of serological results.

It would not be practical for busy hospitals to carry out viable cell counts on all faeces from patients suspected to be suffering from *Cl. welchii* food poisoning. The following technique, however, is suggested as an alternative measure:

1. A thick emulsion of faeces (1/5–1/10) is made in quarter-strength Ringer's solution.

2. With this emulsion a semi-quantitative direct count is carried out on neomycin blood agar using a calibrated loop in a manner similar to that now routinely used in many hospitals for urine culture. This count may be done either on unheated faeces or on the emulsion heated to 80° C. for 10 min. (spore count). The heating method has the advantage that it activates spores (which is more important in outbreaks due to heat resistant *Cl. welchii*) and makes the isolation of *Cl. welchii* a little easier, but it has the disadvantage of being time consuming. The plates are incubated anaerobically at 37° C. for 16–24 hr. (no longer). Using this technique it is at least possible to tell whether *Cl. welchii* are present in the faeces in relatively small or large numbers.

3. One–two ml. of the emulsion is inoculated into a tube of C.M.M. and heated at 100° C. for 60 min. It is then incubated overnight at 37° C. and subcultured on neomycin blood agar. This detects the presence or absence of heat-resistant spores of *Cl. welchii*.

Media routinely used for the isolation of *Salmonella*, *Shigella* and *Staphylococcus* can be inoculated using this emulsion although this must be done before heating.

SUMMARY

Details of confirmed outbreaks of food poisoning due to heat-sensitive *Cl. welchii* are given. In 4/5 incidents heat-sensitive *Cl. welchii* were isolated in large numbers from the majority of the faeces. In the remaining outbreak the faecal samples were not collected until 7–9 days after the illness. The causative organism was isolated from the food in 3/5 instances.

In addition four outbreaks of food poisoning in which both heat-sensitive and heat-resistant *Cl. welchii* were isolated are described.

The role of heat-sensitive *Cl. welchii* in food poisoning outbreaks is discussed and a suggested method of examining faeces for *Cl. welchii* is given.

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