CURRENT DEVELOPMENTS IN DETECTION OF MICROORGANISMS IN FOODS-CLOSTRIDIUM PERFRINGENS

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ABSTRACT

Recent work on Clostridium perfringens foodborne disease has established that any type, whether classical or "food-poisoning," may cause illness if present in sufficiently large numbers. Cultural methods for detection and enumeration of the organism have been suggested, and some of these methods have proven satisfactory in field trials. New approaches, such as the cultural or serological examination of patients' feces or the direct examination of foods for toxin content, have been suggested. The problem has been shown to be of sufficient importance to warrant continued and increased study.

History

The type of foodborne illness that results from the microorganism, Clostridium perfringens, is not a new disease, although most of the work on its detection has been carried out in the last 15 to 20 years. As early as 1895, Klein (21), in England, described outbreaks of foodborne illness caused by an organism that he called Bacillus enteritidis sporogens. His description of the isolates, however, indicates that he was probably dealing with C. perfringens. Over the years, many similar outbreaks have been reported from England by Andrews (1) in 1899, Knox and MacDonald (22) in 1943, Duncan (6) in 1944, and by Hobbs and associates (19) in 1953. In 1933 Nelson (26) reported an outbreak in Fargo, North Dakota, involving infants who had become ill from milk contaminated with dust containing spores of C. perfringens. The first well authenticated outbreaks in the United States, however, were described by McClung (24) in 1945. These outbreaks, which resulted from contaminated chicken and gravy, occurred in 1943, 1944, and 1945. Since McClung's report, many outbreaks have been studied in Great Britain, Europe, Japan, and the United States.

The Illness

Clostridium perfringens food poisoning is a relatively mild disease. After an incubation period of 8 to 22 hr, patients develop acute abdominal pain and diarrhea. Nausea and vomiting are rare, and pyrexia, shivering, headache, and other signs of infection are seldom present. The illness is of short duration, usually one day or less, and is followed by complete and uneventful recovery.

The Causative Organism

This type of foodborne illness is caused by toxico- logical type A strains of C. perfringens. The classical strains of this type that cause gas gangrene in man are characterized by significant production of the alpha and theta toxins and, therefore, produce both complete and partial hemolysis on blood agar. They also produce spores that are sensitive to heating at 100 C for a few minutes, and, serologically, they are almost strain specific. In the years immediately following World War II, Dr. Betty Hobbs and her associates (19) made comprehensive studies of the outbreaks resulting from this organism in Great Britain. They discovered that they were isolating strains of C. perfringens with characteristics that differed from the classical strains. These food isolates have been referred to as the "food poisoning type." They produced low levels of alpha toxin, and little or no theta toxin. Therefore, they were nonhemolytic on blood agar or produced only narrow zones of partial hemolysis. They produced heat resistant spores, many of which would withstand boiling for as long as 1 to 5 hr, and they could be typed serologically using 13 antisera (19).

In the early 1960's researchers in the United States Public Health Service in Cincinnati began studying outbreaks of C. perfringens foodborne illness and isolates from foods and other sources in the United States. From these studies it was concluded that, in this country at least, there was no specific group of C. perfringens strains that could be referred to as "the food poisoning type." Rather, it appeared that foodborne disease was caused by classical strains, the English type, and strains with intermediate charac-
teristics (12). These observations have been substantiated by excellent work in Canada by Dr. Hauschild and his associates (15, 16, 17, 18). These investigators showed that experimental food poisoning could be produced in human volunteers by a food poisoning isolate that produced nonheat resistant spores (17). Furthermore, using lambs as test animals these workers were able to demonstrate experimental enteritis with both classical and food poisoning strains of C. perfringens (19). Using both types of strains (18), they also have produced typical gas gangrene in guinea pigs. Using the "intestinal loop technique," they have demonstrated that C. perfringens cells, but not culture medium, produce reactions and fluid accumulation and that the reactions were not prevented by alpha-antitoxin (16). Similar results also have been obtained by Duncan et al. (8), using the "rabbit ileal loop technique."

The friendly disagreement between English workers and ourselves concerning the characteristics of the causative organisms of C. perfringens foodborne disease has now been resolved. In 1967, Sutton and Hobbs (30) published a description of five outbreaks caused by heat-sensitive strains of C. perfringens in England. It thus becomes mandatory that all of us look for both heat-resistant and heat-sensitive strains when studying such outbreaks.

The question that next comes to mind is quite obvious. What is it about this organism that leads to foodborne disease? The work of Dack (3), Hobbs (19), and Disehe and Elek (4), and most recently Hauschild and Thatcher (17, 18), prove that it is not one or another of the preformed toxins. The clinical syndrome of the illness makes it unlikely that it is a true infection. All work to date indicates that a very high level of viable vegetative cells, several millions per gram, are needed to cause symptoms. Dr. Hauschild in a personal communication states, "Our conclusion is that, although the disease is produced by C. perfringens cells, the food poisoning factor is produced in situ and is probably not a cellular constituent."

It has been postulated that the large number of cells required to produce illness result from mishandling of meats, meat dishes, or gravies. Poor sanitation practices may allow contamination after cooking, and growth of the organism or residual spores may germinate after cooking and grow to substantial levels of contamination. Two recent publications emphasize this latter possibility. Strong and Ripp (29) showed that spores of heat-resistant strains of C. perfringens survived after cooking of hams, turkey rolls, and ground beef casserole. Furthermore, the number of viable spores was only slightly reduced by refrigeration for 48 hr. Mishandling, such as slow cooling or warming after refrigeration, could allow the outgrowth of the organisms. Pivnick et al. (27) showed that the spores of C. perfringens survived in barbecued chicken and that outgrowth to significant levels would occur in 12 to 16 hr at 45 C.

Cultural Determination of Outbreaks

It is not the purpose of this paper to discuss all of the recent publications on various aspects of work on C. perfringens. A few of these, however, seem quite pertinent to our interests.

The sporulation of C. perfringens has been a knotty problem for those interested in studies on the survival of the organism in foods and in time-temperature relationships. Two publications on the problem of spore production have recently appeared. Kim, Che­ney, and Woodburn (20) proposed a new sporulation medium that combined the relatively large spore crops obtained in Ellner's (9) medium with the production of normally heat-resistant spores obtained in SEC broth (2). Their medium contained peptone, trypticase, starch, NaCl, MgSO₄, and thiamine hydrochloride. Incubation at 37 C for 24 hr yielded excellent spore crops. Similarly, Duncan and Strong (7) reported on a medium containing yeast extract, proteose peptone, starch, sodium thioglycollate, and NaHPO₄ that gave superior results. The addition of activated carbon resulted in additional increases in spore numbers and in heat resistance with some strains. It seems possible that the use of these new media may allow investigators to obtain spore crops for much needed studies on spore survival.

Enumeration of C. perfringens in outbreak foods is a necessity in determining the etiology of the disease. Methods for this purpose have appeared in the literature in recent years. Marshall, Steenbergen, and McClung (23) published a report on a rapid technique in which a medium containing tryptone, sulfite, yeast extract, polymyxin, and neomycin was used. These authors utilized an incubation temperature of 46 C and reported the medium to be practically specific for C. perfringens. To my knowledge, the use of this medium in the study of outbreak foods has not been reported. Green and Litsky (10) described a new medium and a Most Probable Number (MPN) technique for enumerating C. perfringens. They reported a significantly higher recovery rate for this method as compared to a plating procedure and suggested that it could be of value in quality control work where the number of C. perfringens is usually quite low. Hall (11) reported the results of a collaborative study of a quantitative method for C. perfringens in foods. This method is essentially the same as that described by Angelotti et al. (2) in 1962. It was adopted as official, first
action, by the Association of Official Analytical Chemists. The method uses the Sulfite-Polymyxin-Sulfadizene (SPS) agar developed by Angelotti et al. (2). Suitable dilutions of a 1:10 blend of the food are plated in duplicate in SPS agar and incubated anaerobically for 24 hr at 35°C. The black colonies appearing on the SPS agar plates are counted and recorded as the total clostridial count. A suitable number of colonies picked to a nitrate-motility medium contains antibiotics and has been used by the New York City Department of Health for the examination of outbreak and other foods.

**Table 1. Serological Typing of C. perfringens Isolates From Outbreaks**

<table>
<thead>
<tr>
<th>Type of patient</th>
<th>Number of patients tested</th>
<th>Type of C. perfringens in food</th>
<th>Type of C. perfringens in patients</th>
<th>Frequency of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9</td>
<td>None</td>
<td>PS35, PS43, PS14</td>
<td>2</td>
</tr>
<tr>
<td>Outbreak 1</td>
<td>14</td>
<td>(PS72, PS76, PS81A)</td>
<td>(PS73, PS76, PS81A)</td>
<td>12</td>
</tr>
<tr>
<td>Outbreak 2</td>
<td>38</td>
<td>PS89</td>
<td>PS99</td>
<td>24</td>
</tr>
<tr>
<td>Outbreak 3</td>
<td>6</td>
<td>None</td>
<td>(PS66, PS88)</td>
<td>6</td>
</tr>
<tr>
<td>Outbreak 4</td>
<td>10</td>
<td>None</td>
<td>Hobbs 12</td>
<td>7</td>
</tr>
<tr>
<td>Outbreak 5</td>
<td>11</td>
<td>PS55, H9, H13</td>
<td>PS24</td>
<td>6</td>
</tr>
<tr>
<td>Outbreak 6</td>
<td>22</td>
<td>H12 (PS66, PS88)</td>
<td>H12</td>
<td>16</td>
</tr>
<tr>
<td>Outbreak 7</td>
<td>15</td>
<td>PS90, PS75</td>
<td>PS40, H13, H3</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 2. Cases of Foodborne Illness of Bacterial Origin Reported in 1968 (25)**

<table>
<thead>
<tr>
<th>Bacterial</th>
<th>Number</th>
<th>Per cent</th>
</tr>
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<tbody>
<tr>
<td>C. perfringens</td>
<td>5,966</td>
<td>40.8</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>4,149</td>
<td>30.2</td>
</tr>
<tr>
<td>Salmonella</td>
<td>1,287</td>
<td>8.8</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>1,234</td>
<td>8.8</td>
</tr>
<tr>
<td>E. coli</td>
<td>1,234</td>
<td>8.4</td>
</tr>
<tr>
<td>Shigella</td>
<td>407</td>
<td>2.8</td>
</tr>
<tr>
<td>Brucella</td>
<td>12</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>C. botulinum</td>
<td>10</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14,017</td>
<td></td>
</tr>
</tbody>
</table>

**Other Approaches to Determination of Outbreaks**

As all those involved in the study of foodborne disease outbreaks know, there are many occasions when no suitable food is available for examination. Either the food has been discarded, or it has been refrigerated, frozen, or mishandled. Because of this, other approaches to the determination of a C. perfringens etiology have been suggested. It has been shown, by using enrichment techniques and resampling, that practically 100% of human fecal specimens contain C. perfringens. In normal individuals not exposed to outbreak conditions, the level of C. perfringens in the feces may be quite low, but, following an outbreak, these levels rise significantly. Dr. Sutton and Dr. Hobbs in England have suggested a simple method of evaluating this situation. A 1:10 suspension of feces is streaked on blood agar containing neomycin in such a manner that three successive streaks are made from one original small drop. The plates are incubated anaerobically at 46°C. If the patient has been recently exposed to a C. perfringens outbreak, there will be over 10 colonies in the third streak, indicating a level of about 10⁹ organisms per g. Normal individuals, on the other hand, will show growth of C. perfringens only in the first streak. The very simplicity of this method indicates that it deserves some trial to determine its usefulness.

As was mentioned earlier, foods for examination may be refrigerated or frozen before receipt by the laboratory. This can reduce the numbers of viable C. perfringens by as much as 2 or more logs, thus yielding low results that might be interpreted as
reached levels of 1.8 to 2.3 million cells per gram of food. For the presence of lecithinase. Using either outbreak foods.

being below the level necessary to cause disease. Harmon (14) has suggested a method of testing such foods for the presence of lecithinase. Using either the hemolysin indicator plate or the lecithovitellin test, he was able to detect the alpha toxin (lecithinase) when the viable count of C. perfringens had reached levels of 1.8 to 2.3 million cells per gram of food. Such levels and higher are usually found in outbreak foods. It would seem, therefore, that the method has merit for the examination of foods and would allow the determination of a C. perfringens etiology when the viable count has been reduced by freezing or subsequent reheating.

Another approach to the determination of an outbreak of C. perfringens foodborne disease is the serological typing of isolates obtained from the food and the feces of patients. Furthermore, in those instances in which no food is available for examination, such studies on cultures from the feces of the patients appear to yield equally valid information. If one makes 3 to 4 isolates from the feces of each of a group of normal nonexposed individuals, one can expect that about 10 to 20% of the individuals will be carrying the same serotype. If, on the other hand, a group of individuals have had a common source of exposure to large numbers of a single strain of C. perfringens, as occurs in outbreak conditions, one or more of the isolates from most of the individuals will be of the same serotype.

At the present time, a cooperative study is being conducted by the Anaerobic Laboratory of the National Communicable Disease Center in Atlanta and the Milk and Food Sanitation Research of the Division of Microbiology in Cincinnati (13). The NCDC laboratory is studying the serologic properties of strains from outbreaks and our laboratory is examining the cultural characteristics of the same strains. A comprehensive study of the serotypes is being made with the use of over 90 antisera prepared at NCDC (5). Some representative results are shown in Table 1. It can be noted that the first line shows the results with 9 normal nonexposed individuals. Three serotypes—PS35, PS49, and PS14—occurred more than once. In each instance, the serotypes were detected in the feces of two individuals, which indicated that 22.2% were carrying the same serotypes. In the first outbreak, a strain that reacted to three sera—PS72, PS76, and PS81A—was found in the food. This same strain occurred in the feces of 12 of the 14 individuals, or 85.7%. This indicates a common source of fecal contamination. Outbreak two was a very large one, involving over a thousand people. Data on isolates from 38 of those who became ill are shown. The food contained serological type PS89, as did 24, or 63.1%, of the fecal specimens of the ill patients. This percentage appears rather low, but some of the specimens were obtained nearly 2 weeks after the outbreak. Six patients were studied from outbreak 3, but no food was available. All six of the fecal specimens contained a strain reacting with PS66 and PS88 antisera, again indicating a common source. Outbreak 4 is similar. No food was available, but 7 of the 10 patients, 70%, were carrying Hobbs type 12 C. perfringens.

Outbreak 5 is interesting in that the food examined contained three serotypes PS55, Hobbs 9, and Hobbs 13. Six of the patients, on the other hand, were carrying serotypes PS24. It is suspected that in this outbreak a different food served as the source of the organism. Outbreak 6 is the first one that we have encountered in which the food contained two serotypes that were also found in the feces of the patients. Hobbs strain 12 occurred in 16 of 25 patients, or 72.7%, and the strain reacting to PS66 and PS88 occurred in 4 or 18.1% of the patients. Outbreak number 7 gave inconclusive results. The food, roast beef, contained two serotypes, PS90 and PS75. The fecal specimens from 15 patients gave reactions to at least 18 serotypes. Eight of these occurred more than once, but none occurred in more than two individuals. The result was a frequency of occurrence of only 13.3%. It seems doubtful that this group received their C. perfringens strains from a single source.

A total of 20 suspected outbreaks have been studied to date, and in 13, or 65.0%, of them it has been possible to determine that a common source of C. perfringens was present.

Regardless of the methods that may be used to determine that outbreaks of C. perfringens foodborne disease are occurring, it is obvious that such determinations must be made if a complete picture of the incidence of such disease in a country is to be seen. Table 2 shows the number and percentage of cases of foodborne illness of bacterial origin reported in the United States in 1968 (25). Clostridium perfringens tops the list with 5,966 cases or 40.8% of the total of 14,617 cases. The staphylococci caused 4,919 or 30.2% of the total and were second on the list. Salmonella (1287), streptococci (1282) and E. coli (1234) were very similar in their levels and accounted for 8.8, 8.8, and 8.4%, respectively. Shigella accounted for 407 cases or 2.8% of the total while the brucellae and C. botulinum with 12 and 10 cases accounted for less than 1%.

REFERENCES

13. Hall, H. E., and V. R. Dowell. 1967. Foodborne disease outbreaks caused by Clostridium perfringens. Distributed by the National Center for Urban and Industrial Health and the National Communicable Disease Center, PHS, DHEW, as a part of a packet of materials related to a cooperative study of Clostridium perfringens isolates.