Incidence of *Clostridium perfringens* in American Foods

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**Abstract**

Strong, Dorothy H. (University of Wisconsin, Madison), James C. Canada, and Bertie B. Griffiths. Incidence of *Clostridium perfringens* in American foods. Appl. Microbiol. 11:42–44. 1963.—Food samples were examined for the presence of *Clostridium perfringens*. A medium described by Mossel and later modified by Angelotti et al. was used for the detection and enumeration of *C. perfringens*. The incidence of *C. perfringens* observed in the foods examined was 6.1%. *C. perfringens* was recovered from 2.7% of the commercially prepared frozen foods, 3.8% of fruits and vegetables, 5.0% of spices, 1.8% of home-prepared foods, and 16.4% of raw meat, poultry, and fish.

*Clostridium perfringens* has found considerable acceptance as a probable etiologic agent of food poisoning. Reports incriminating this organism as the causative factor of food-borne illnesses have been published in the British Isles (Hobbs et al., 1953; Dische and Elek, 1957; McKillop, 1959), Germany (Linzenmeier, 1956; Adam, 1958), Hungary (Nikodemusz and Felix, 1960), Poland (Meisel, Toembowlen, and Pogorzelska, 1961), Sweden (Osterling, 1952), Denmark (Dam-Mikklesen, Petersen, and Skovgaard, 1962), Japan (Hoyashi et al., 1961), Canada (Fearnough, personal communication) and the United States (McClung, 1945; McClung and Fleming, 1961; Anonymous, 1961, 1962). Symptoms of the poisoning, as reported by Hobbs et al. (1953), McClung (1945), and Smith (1955), include diarrhea, acute abdominal pain, nausea, and occasional vomiting. These symptoms are first noticed 8 to 12 hr after ingesting the food and continue for 6 to 12 hr. A fatal case of poisoning attributed to *C. perfringens* was reported by Kellert and Meller (1953).

From the literature published, it appears that *C. perfringens* may be an inhabitant of many and various foods. McClung and Fleming (1961) found the microorganism in cooked shrimp. Yamamoto et al. (1961) recovered *C. perfringens* from uncooked poultry. It has been found in cooked chicken by McClung (1945) and by McKillop (1959), in raw vegetables by Burzynska (1955), in dairy products and eggs by Nikodemusz and Felix (1960), in meats by Hobbs (1960), Hobbs and Wilson (1959), and Wisniewski (personal communication). In addition, the organism has been isolated from soils (Smith and Gardner, 1949) and from feces of healthy human beings (Meisel et al., 1961; Hobbs et al., 1953).

It was felt that information concerning the incidence of *C. perfringens* in American foods commonly available to the consumer or prepared by homemakers would be helpful in assessing the problem in the United States.

**Materials and Methods**

*Sample collection and preparation.* In the study, 510 food samples were examined for the presence of *C. perfringens*. The samples were divided into five different food groups: commercially prepared frozen foods, fresh vegetables and fruits, spices, home-prepared foods, and raw meats, poultry, and fish. The laboratory procedure for the detection and enumeration of *C. perfringens* was essentially the same for all groups and followed closely a plan recommended by Hall (personal communication).

The frozen foods were meat or poultry dishes, with accompanying gravies or sauces. The foods were purchased from a variety of retail outlets. The samples remained in the frozen state from the purchase time to that of laboratory sampling. Approximately 25 g of food were removed from the intact frozen sample by the use of either a sterile cork-borer or knife blade. The portion so removed was diluted 1:10 in sterile water, blended for 2 min at slow speed, and then diluted further; 1-ml samples of the various dilutions were plated in duplicate. Angelotti et al. (1962) recommended that the plates be incubated for 24 hr in anaerobic jars (Anaerojar, Case Laboratories, Inc., Chicago, Ill.) in an atmosphere of 90% nitrogen and 10% carbon dioxide (The Matheson Co., Inc., Joliet, Ill.). This procedure was followed regularly, although in some instances the incubation time was continued to a maximum of 72 hr.

The fresh vegetables and fruits were refrigerated in the laboratory only if they had been refrigerated at the time of retail purchase. Sections of the sample were made with a sterile blade on the unwashed food.

The spices had been commercially ground and packaged; 1-g samples were diluted to the desired concentrations in sterile phosphate solution (American Public Health Association, 1960).

The home-prepared foods were purchased from home-
makers residing in Madison, Wis. The homemakers were selected in such a way as to represent all geographical areas in the city. The field worker purchased a portion of the meat dish, including accompanying gravies and sauces being served at the meal. The dates and times that the samples would be collected were unknown to the homemakers. The samples were collected aseptically and transported to the laboratory under refrigeration. The foods were examined in the laboratory within 1 hr after collection.

The samples of meat, poultry, and fish were purchased from retail markets. Temperature of the foods at the time of purchase was maintained until laboratory sampling. Some of the foods were precut and wrapped and others were cut and wrapped at the time of purchase. The blended samples were diluted in 0.1% peptone-water and then plated.

_C. perfringens isolation and confirmation_. A medium described by Mossel et al. (1956) and Mossel (1959) and later modified by Angelotti and Hall (1961) and Angelotti et al. (1962) was used to isolate and enumerate _C. perfringens_. When grown in this medium, clostridia produce black colonies due to their ability to reduce sulfite to sulfide in the presence of iron. Other microorganisms are inhibited or do not produce black colonies.

Black colonies were confirmed as _C. perfringens_ by the criteria shown in Table 1. Sugar-fermentation reactions were obtained by adding 1% levels of the desired sugar to sugar-free thioglycolate medium. The sugar media were inoculated with 0.5 ml of a 4-hr culture of the isolate grown in sugar-free thioglycolate medium. A medium suggested by Angelotti et al. (1962) was used to determine motility and nitrate reduction simultaneously. A positive lecithovitellin reaction was demonstrated by using an egg-yolk medium (McClung and Toabe, 1947). Observations for double zones of hemolysis, which are characteristic of _C. perfringens_ when grown on bovine blood agar (Yamamoto et al., 1961), were made on both rabbit blood agar and human blood agar. At this date, no attempts have been made to type the isolates.

A culture of _C. perfringens_ (NCTC 8799, F. 1546/52) was obtained from B. C. Hobbs, Public Health Laboratory Service, London, England. This culture had originally been isolated from a food-poisoning outbreak and served as a control for all sample-plating experiments. Stock cultures were maintained in veal broth and transferred monthly.

**RESULTS**

Results obtained from examining the foods by the preceding methods are presented in Table 2.

The frozen foods included TV dinners, meat and poultry pies, and similar products. The three samples found positive from this group were obtained from frozen turkey pies.

Twenty types of spices were examined. The positive samples obtained were paprika, savory, and poultry seasoning.

The home-prepared foods had the lowest incidence rate of the foods examined. The composition and preparation of these foods varied as would be expected. Canned corn beef, bologna, and a dish composed of canned beans and hamburger provided the positive samples.

The highest incidence rate was found in the meat, poultry, and fish group. The samples included 26 beef cuts, 23 pork cuts, 5 lamb cuts, 3 veal cuts, 28 spiced meats, 14 organ meats, 9 ground meats, 11 fish samples, and 3 samples of chicken. _C. perfringens_ was recovered from two beef cuts, one cut of pork, two cuts of lamb, four samples of ground meats, five samples of spiced meats, and six samples of organ meats.

Of 510 foods sampled, 31 demonstrated the presence of _C. perfringens_. The incidence of the total was 6.1%.

Black colonies appearing on the medium proposed by Angelotti et al. (1962) were routinely counted and recorded at the end of 24 hr. When plate counts were low, all black colonies were picked and subjected to the confirmatory tests shown in Table 1. In addition, slides were prepared from the colony growth. When the Gram stain was applied and the slides examined microscopically, the cells from those colonies which subsequently were confirmed as _C. perfringens_, in all cases, appeared to be short, thick rods which were gram-positive.

In those situations in which larger numbers of black colonies were encountered, the colony from which the isolate was to be obtained was identified and sent to the Public Health Laboratory, London, England. In the case of positive results, the type was confirmed by the National Collection of Type Cultures, London.

**TABLE 1. Criteria for confirmation of Clostridium perfringens**

<table>
<thead>
<tr>
<th>Characteristic or medium</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron litmus milk</td>
<td>Stormy fermentation</td>
</tr>
<tr>
<td>Glucose</td>
<td>Acid, gas</td>
</tr>
<tr>
<td>Maltose</td>
<td>Acid, gas</td>
</tr>
<tr>
<td>Lactose</td>
<td>Acid, gas</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Acid, gas</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>No acid, no gas</td>
</tr>
<tr>
<td>Salicin</td>
<td>No acid, no gas</td>
</tr>
<tr>
<td>H2S</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
</tr>
<tr>
<td>Lechithinase</td>
<td>+</td>
</tr>
<tr>
<td>Blood agar</td>
<td>Beta-hemolysis</td>
</tr>
<tr>
<td>Motility</td>
<td>Nonmotile</td>
</tr>
<tr>
<td>Gram stain</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**TABLE 2. Incidence of Clostridium perfringens in foods**

<table>
<thead>
<tr>
<th>Food group</th>
<th>No. of samples positive</th>
<th>Range of count (cells/g)</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially prepared</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>frozen foods</td>
<td>111</td>
<td>10–20</td>
<td>2.7</td>
</tr>
<tr>
<td>Raw fruits and vegetables</td>
<td>52</td>
<td>10–140</td>
<td>3.8</td>
</tr>
<tr>
<td>Spices</td>
<td>60</td>
<td>10–30</td>
<td>5.0</td>
</tr>
<tr>
<td>Home-prepared foods</td>
<td>165</td>
<td>10–1,180</td>
<td>16.4</td>
</tr>
<tr>
<td>Meat, poultry, and fish</td>
<td>122</td>
<td>10–1,180</td>
<td>16.4</td>
</tr>
</tbody>
</table>
colonies appeared, representative colonies were selected and similarly treated. When black colonies could not be confirmed as *C. perfringens*, the food samples were recorded as negative. Plates on which only nonblack colonies appeared on prolonged incubation were regarded as negative.

While some black colonies, other than *C. perfringens*, do grow on the medium, the experienced observer finds little difficulty in recognizing *C. perfringens* colonies. In general, the colonies are round to oval in shape and tend to appear slightly thickened at the center. The edge is regular.

**DISCUSSION**

It is evident from the data presented that *C. perfringens* may exist in foods purchased and in foods prepared by consumers. The observed incidence rate is essentially in accordance with that reported by McKillop (1959) and Hobbs et al. (1953). The fact that *C. perfringens* is found in fewer foods prepared by homemakers than in uncooked foods suggests that the preparation process sometimes kills the organism. The isolates have not yet been typed by any technique which indicates the nature of toxin production, nor have other procedures been employed which might indicate the degree of pathogenicity. Studies concerning the tolerances of *C. perfringens* to lowered and elevated temperature extremes are being conducted and will be reported at a later date. Black colonies were always recoverable from the Hobbs strain of *C. perfringens* after an incubation time of 24 hr. Some isolates from foods, however, were not detected until after 48 hr of incubation. Mossel (1959) used an incubation time of 2 to 5 days when purifying newly isolated cultures of *C. perfringens* in pored plates of sulfite-polymyxin-agar.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


