Outbreaks of food-poisoning associated with lecithinase-negative *Clostridium perfringens*

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**Summary.** *Clostridium perfringens* type A is a common cause of food-poisoning. Production of lecithinase (α toxin) is frequently used to identify the organism. Details of 10 outbreaks of food-poisoning caused by lecithinase-negative *C. perfringens* are reported here.

**Introduction**

The vehicles of infection in *Clostridium perfringens* food-poisoning are predominantly meat and poultry or their products which have been inadequately cooked or stored incorrectly after cooking, or both. Surviving spores germinate and vegetative cells multiply rapidly in the food. Large numbers of cells are then ingested; these sporulate, release enterotoxin in the small intestine and cause abdominal pain and diarrhoea.\(^1,2\) Criteria for implicating *C. perfringens* as the cause of an outbreak include isolation of strains with the same serotype from a proportion of faecal specimens, and from food if available, and the detection of enterotoxin in faeces.\(^1,2\)

Production of lecithinase, or α toxin, together with complete neutralisation of the toxin by a specific antiserum (the Nagler reaction) is commonly used to identify *C. perfringens* and to differentiate it from other lecithinase-producing clostridia which may be present in faeces, such as *C. absomum, C. baratti, C. bifermentans, C. sordelii* and *C. centurosporogenes,* where lecithinase activity is only partially neutralised by antiserum specific for *C. perfringens*.\(^3\) However, some strains of *C. perfringens* do not produce lecithinase and organisms which were previously called *C. plagaryum* have been reclassified as lecithinase-negative variants of *C. perfringens*.\(^3\)

The Food Hygiene Laboratory (FHL) of the Public Health Laboratory Service (PHLS) investigates c. 150 incidents of suspected *C. perfringens*-associated diarrhoea each year and confirms 40–90 outbreaks of *C. perfringens* food-poisoning. Ten outbreaks of food poisoning caused by lecithinase-negative *C. perfringens* have occurred since 1974 and are reported here.

**Materials and methods**

**Strains of *C. perfringens* and their enumeration**

Strains of *C. perfringens* isolated from faeces and foods in food-poisoning outbreaks were sent to FHL from PHLS and NHS laboratories in the UK. The number of cfu of *C. perfringens*/g of food was estimated by the referring laboratory in three outbreaks.

**Serotyping**

All strains were plated on neomycin blood agar, incubated anaerobically for 18–24 h at 37°C and the pattern of haemolysis was recorded. Colonies were subcultured to egg yolk medium, half the plate having been spread with specific *C. perfringens* type A antitoxin (Wellcome) and the Nagler reaction was recorded after anaerobic incubation for 18–24 h at 37°C.\(^4\) Colonies were also subcultured on to Columbia blood agar (CBA) and incubated anaerobically at 37°C overnight. If the strain was a pure culture on CBA, serotyping was performed by a slide agglutination method.\(^5,4\)

**Biochemical tests**

The API rapid ID32A (bioMérieux SA, Lyon, France) was used according to the manufacturer's instructions. Briefly, overnight growth on Columbia blood agar was resuspended in 2 ml of sterile water to an optical density of 4 McFarland units. Each cupule was inoculated with 55 μl of cell suspension, incubated anaerobically for 4 h and then read visually.

**Faeces**

One volume of faeces was mixed with up to four volumes of phosphate-buffered saline, pH 7.3, and
Table. Outbreaks of food-poisoning associated with lecithinase-negative *C. perfringens* 1974–1993

<table>
<thead>
<tr>
<th>Date</th>
<th>Serotype in patients' faeces or food (number positive/number tested)</th>
<th>Haemolysis</th>
<th>Community affected</th>
<th>Food incriminated</th>
<th>Symptoms, onset</th>
<th>Number ill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 1974</td>
<td>27 (15/16 pt)</td>
<td>α,β</td>
<td>Hospital</td>
<td>Roast lamb+ gravy?</td>
<td>&quot;food poisoning&quot;</td>
<td>105 + ?</td>
</tr>
<tr>
<td>Aug. 1975</td>
<td>25.27 (1/2 pt)</td>
<td>&quot; + ve &quot;</td>
<td>Luncheon club</td>
<td>Steak and kidney</td>
<td>D 12 h</td>
<td>18</td>
</tr>
<tr>
<td>July 1976</td>
<td>25.27,31,68 (9/9 pt)</td>
<td>α,β</td>
<td>Geriatric hospital</td>
<td>Roast beef</td>
<td>D 12–15 h</td>
<td>68</td>
</tr>
<tr>
<td>Jan. 1977</td>
<td>25.27,31,68 (4/6 pt)</td>
<td>α,β</td>
<td>Geriatric hospital</td>
<td>Minced beef</td>
<td>D 12 h</td>
<td>6 + ?</td>
</tr>
<tr>
<td>Oct. 1977</td>
<td>25.27,31,68 (8/8 pt)</td>
<td>α,β</td>
<td>Hospital</td>
<td>?</td>
<td>AP, D</td>
<td>8 + ?</td>
</tr>
<tr>
<td>Aug. 1978</td>
<td>25.27,31,68 (32/34 pt)</td>
<td>α,β</td>
<td>Hospital</td>
<td>Chicken</td>
<td>D, (V) 6–24 h</td>
<td>106</td>
</tr>
<tr>
<td>Feb. 1982</td>
<td>NT (6/6 pt)</td>
<td>&quot; + ve &quot;</td>
<td>Old people's home</td>
<td>Mutton</td>
<td>V, D 1 h</td>
<td>9</td>
</tr>
<tr>
<td>June 1985</td>
<td>27 (3/3 pt)</td>
<td>&quot; + ve &quot;</td>
<td>Training centre</td>
<td>Roast lamb</td>
<td>AP, D 9–16 h</td>
<td>12</td>
</tr>
<tr>
<td>Dec. 1992</td>
<td>27 (4/5 pt)</td>
<td>θ</td>
<td>Nursing home</td>
<td>Mince?</td>
<td>D 12 h</td>
<td>4</td>
</tr>
</tbody>
</table>

AP, abdominal pain; D, diarrhoea; V, vomiting; pt, patients; fd, food.

clarified by centrifugation at 12000 *g* for 20 min. The supernate was filtered through a 0.8-μm and a 0.2-μm membrane filter in series and then tested for enterotoxin by reversed passive latex agglutination (PET-RPLA, TD930, Unipath). A difference of two or more wells between the sensitised latex and the control latex was classified as a positive result for enterotoxin.

**Results**

The first outbreak of food poisoning caused by lecithinase-negative *C. perfringens* in the UK occurred in 1974. Ten outbreaks have now been investigated by FHL (table).

The colonial morphology of all outbreak strains was typical of *C. perfringens*. Representative isolates of each outbreak strain were identified as *C. perfringens* by the pattern of end-products of metabolism detected by gas liquid chromatography (GLC) at the PHLS Anaerobe Reference Unit. Four strains from the most recent outbreak were tested in the API rapid ID32 by FHL. All were identified as *C. perfringens*. An outbreak strain from the August 1975 and the December 1976 incidents were also tested: both were identified as *C. perfringens*. Strains from the other outbreaks were no longer available for testing.

The number of organisms in incriminated foods was estimated in three outbreaks and was between 1.5 x 10⁶ and 1.1 x 10⁷ cfu/g of food. Eight of the 10 outbreaks were caused by strains with antigen 27 or 27 in combination with other antigens (table). Serotype 27 has been implicated in 12 outbreaks of food poisoning in the period 1974–1993, the strains being lecithinase-negative on two occasions. Serotypes 25,27 and 25,27,31,68 have occurred only among lecithinase-negative strains. In contrast, between 1974 and 1993, serotype 11,13 has been implicated in a total of 35 outbreaks and only one strain was lecithinase-negative.

Isolates from the first three outbreaks (1974–1976) have been shown to produce enterotoxin *in vitro*. Four faecal specimens from the most recent outbreak were examined; enterotoxin was detected in one patient.

All 10 outbreaks were caused by meat or poultry cooked on a large scale and, with one exception, the symptoms (predominantly diarrhoea and abdominal pain) and their onset (8–24 h) were typical of *C. perfringens* food-poisoning. In a typical outbreak, in August 1978, in a hospital, sixty chickens weighing 2 lb each were defrosted for 3 h at room temperature, cooked in a single batch for 3 h in a boiler, cooled for 1 h at room temperature, and then stored at 40°F for 16.5 h. During this period they were deboned at room temperature. Portions were warmed in a trolley by adding hot gravy made with chicken and beef bones. Of 130 patients, 106 suffered diarrhoea 6–24 h later.

**Discussion**

These outbreaks show that reliance on detection of lecithinase production for identification of *C. perfringens* will miss some strains and hence some outbreaks. After an outbreak of food-poisoning with a clinical history suggestive of *C. perfringens* (abdominal pain and diarrhoea 8–18 h after eating meat or meat products), organisms with a colonial morphology typical of *C. perfringens* should not be dismissed as non-pathogenic if they are apparently lecithinase-negative on egg-yolk medium. The strains described here all had a colonial morphology typical of *C. perfringens* on blood agar, the most common being large smooth slightly opaque colonies, although occasionally rough colonies with a lobate edge are seen. Other colonial morphologies occur less frequently: flat colonies with an irregular surface and filamentous margins and dwarf colonies. On rabbit, sheep, cow, horse or human blood, colonies are non-haemolytic, or with extensive, incomplete, dark edged
haemolysis caused by $\alpha$ toxin or with clear and complete beta-haemolysis due to $\theta$ toxin or with both $\alpha$ and $\theta$ haemolysis.

References


The work of everyone who isolated the organisms and referred them to FHL and all those who performed the serotyping in FHL and so made this paper possible is gratefully acknowledged. I also thank Dr R. Gilbert for his helpful comments.


