Diagnostic Importance of *Clostridium perfringens* Enterotoxin Analysis in Recurring Enteritis among Elderly, Chronic Care Psychiatric Patients

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A series of *Clostridium perfringens*-related gastrointestinal outbreaks occurred over a period of several months among elderly, chronic care patients in a psychiatric hospital. Several serotypes of *C. perfringens* and many nontypeable isolates were found. The distribution of certain serotypes and the incidence of detection of enterotoxin in fecal extracts were related to wards on which patients were resident (six wards were involved). Several patients were reported to have chronic or recurring fecal incontinence or diarrhea or both. With a background of elevated spore counts of several serotypes and chronic diarrhea, only detection of enterotoxin could provide definitive evidence of *C. perfringens* etiology in gastroenteritis cases.

*Clostridium perfringens* is a common bacterial agent of foodborne disease. Symptoms—predominantly diarrhea and abdominal cramps—usually appear 6 to 20 h after ingestion of contaminated food and last for 12 to 24 h after onset. Often, foods suspected of causing an outbreak are not available or are negative for *C. perfringens*. Implication of *C. perfringens* as an etiological agent under such circumstances relies on one or both of the following currently accepted laboratory criteria (2) applicable to stool specimens: (i) the median spore count in ill individuals is ≥10^6/g, or (ii) the same serotype is isolated from most ill individuals. Once the etiology of the outbreak is determined, individuals may be related to the outbreak on the basis of isolated serotypes and epidemiological evidence.

It has been reported, however, that elderly patients in chronic care facilities may be free of enteritis symptoms but carry high numbers of *C. perfringens* cells (6, 7; (M. F. Stringer and R. J. Gilbert, J. Appl. Bacteriol. 51:xvi, 1981)) of certain related serotypes (Stringer and Gilbert, J. Appl. Bacteriol., 1981). If a diarrheal outbreak occurred in such a situation, the traditional criteria described above might have limited applicability, and to interpret the significance of elevated *C. perfringens* spore counts, it might be necessary to analyze stool specimens for *C. perfringens* enterotoxin, i.e., the agent causing clinical symptoms (5). This paper describes the application of *C. perfringens* serotyping and enterotoxin detection as diagnostic tools in a series of diarrheal episodes recurring over a period of several months among elderly, chronic care patients in a psychiatric hospital. The evidence presented illustrates that detection of enterotoxin may be not only a valuable adjunct to existing methodology, but also the only definitive method for diagnosis of *C. perfringens* enteritis cases under certain circumstances.

MATERIALS AND METHODS

**Enumeration of *C. perfringens***. Food samples (25 g) were suspended with 225 ml of buffered peptone by using a stomacher (Colworth 400). Stool specimens (1 g) were suspended in 9 ml of buffered peptone, mixed, and heated for 10 min in an 82°C water bath. Appropriate dilutions of foods and stool specimens were made, and pour plates of tryptose-sulfite-cycloserine medium (3) were prepared (1 ml of each dilution per plate) and incubated anaerobically overnight. Black colonies were counted, and five isolates were selected for confirmation by using lactose-gelatin and nitrate motility media (3). Food samples analyzed included gravy bases, prepared gravy, poultry dressing, meats, vegetable puree, stews, shepherds pie, soups, and custards. Foods served on weekends were most suspect, as enteritis outbreaks occurred primarily on weekends. To optimize the possibility of locating a source, duplicate meals were prepared on one weekend, with one meal served and the duplicate refrigerated. Samples were also taken directly from the serving carts just before the meals were served.

**Serotyping**. A slide agglutination procedure was used to test *C. perfringens* isolates (from pure cultures on blood agar). Rabbit antisera were raised in our laboratory, primarily with reference serotype strains obtained from the National Collection of Type Cultures, London (4). Certain isolates not typeable by our antisera were forwarded to M. F. Stringer in London for typing.

**Enzyme immunoassay for enterotoxin**. Materials for the preparation and assay of immunoglobulin G, immunoglobulin G-alkaline phosphatase conjugate, and enterotoxin were obtained from Sigma Chemical Co. Stool specimens were shipped on ice to the Toronto laboratory from the regional laboratory either directly or after bacteriological analysis. Specimens were extracted with phosphate buffer, and extracts were tested for enterotoxin by a double-antibody sandwich enzyme immunoassay (5), which has an operational specificity of 98.7% and a sensitivity of 93.7% for *C. perfringens* enteritis detection (Jackson, Yip-Chuck, and Brodsky, submitted for publication).

RESULTS

Stool specimens or *C. perfringens* isolates or both were received from 46 patients and 18 staff members over a
7-month period. Multiple specimens from certain patients were submitted as symptoms reappeared. Enzyme immunoassay analysis was carried out on 153 specimens. Serotyping was done on 425 *C. perfringens* isolates.

Figure 1 shows the distribution by month of specimens with elevated *C. perfringens* spore counts (≥10⁶/g) and the number of these specimens in which enterotoxin was detected (percentage of the total with enterotoxin is indicated). The peak incidence of elevated numbers and enterotoxin detection in such specimens was coincident with the peak incidence of enteritis. Most (95%) of the enterotoxin-positive specimens showed *C. perfringens* spore levels of ≥10⁶/g.

Over the course of the outbreaks, specimens were received from patients in six different hospital wards. When the percentage of enterotoxin-positive specimens on each ward was compared with the overall positive rate during the peak period of enteritis (August to October), it became evident that four of these wards (A through D) were primarily involved, with ward D having the greatest number of positive specimens (Table 1). These data (Table 1) include all specimens from patients on wards A through D who were tested for enterotoxin (irrespective of spore count).

Several serotypes were found throughout the course of the outbreaks. Figure 2 shows the incidence of various serotypes. Serotypes 3, 28, and 34 predominated. Many isolates were not typeable by our antisera. Serotypes 83SP108, 83SP225, and 83SP326 (108, 225, and 326 in Fig. 2) were designated by antisera raised in our laboratory to selected nontypeable isolates from the outbreaks. When serotyping data were related to wards, an interesting pattern emerged. All four patients (11 specimens) carrying serotype 34 were resident on ward B. Enterotoxin was not detected in any of these specimens, yet all specimens showed *C. perfringens* spore counts of >10⁶/g. The majority of individuals (6 of 10) carrying serotype 3 (10 individuals, 12 specimens) were resident on ward D. The incidence of serotype 28 also appears to be ward related. Of individuals carrying serotype 28, 40% were on ward D, and 36% were on ward C. One staff member (involved in kitchen cleanup) carried high numbers of serotype 28 spores (as did his spouse). Both had reported illness prior to the outbreaks. However, the staff member also reported symptoms during the outbreak, suggesting that a carrier situation was not present.

![Fig. 1. Relationship between elevated *C. perfringens* spore counts and enterotoxin detection in recurring enteritis outbreaks in the chronic care facilities of a psychiatric hospital.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of Faecal Specimens with spore count ≥10⁶/g</th>
<th>Number positive for enterotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>JUN</td>
<td>80%</td>
<td>59%</td>
</tr>
<tr>
<td>JUL</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td>AUG</td>
<td>59%</td>
<td>29%</td>
</tr>
<tr>
<td>SEP</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>OCT</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>NOV</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>DEC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1. Distribution of enterotoxin-positive specimens on wards A through D**

<table>
<thead>
<tr>
<th>Ward</th>
<th>% of samples enterotoxin positive (no. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 (1)</td>
</tr>
<tr>
<td>B</td>
<td>33 (3)</td>
</tr>
<tr>
<td>C</td>
<td>33 (6)</td>
</tr>
<tr>
<td>D</td>
<td>77 (17)</td>
</tr>
<tr>
<td>A through D</td>
<td>59 (27)</td>
</tr>
</tbody>
</table>

*One of two specimens received in August from ward E was positive for enterotoxin. No other specimens were received from wards E and F during this peak of enteritis.*
All foods tested showed <10 _C. perfringens_ organisms per g.

**DISCUSSION**

The presence of high levels of _C. perfringens_ spores in fecal specimens (median count, \( \geq 10^5 \) g) of individuals with enteroitis compatible with _C. perfringens_ etiology usually provides laboratory confirmation of _C. perfringens_ involvement (2). However, because elderly patients in chronic care facilities may carry high numbers of spores (6, 7; Stringer and Gilbert, J. Appl. Bacteriol., 1981), outbreaks in such situations should be investigated more critically (S. G. Jackson, D. A. Yip-Chuck, and M. H. Brodsky, Abstr. Annu. Meet. Can. Soc. Microbiol. 1984, abstr. no. EM6p). The incidence of elevated counts did, in fact, parallel the pattern of enteroitis occurrence (Fig. 1).

Enteroxin analysis, however, revealed that the situation was more complex. Although a significant number of specimens with elevated spore counts were positive for enteroxin (Fig. 1), the percentages were considerably lower than those of a "typical" _C. perfringens_ restaurant-related incident (Jackson et al., Abstr. Annu. Meet. Can. Soc. Microbiol. 1984). In that incident, 92% of individuals (85% of specimens) with fecal spore counts \( \geq 10^5 \) g were positive for enteroxin. The enzyme immunoassay system has an operational sensitivity of 93.7% for individuals (87.4% for individual specimens) with _C. perfringens_ enterotoxin (Jackson et al., submitted). These facts suggest that the lower percentage of enteroxin-positive specimens with high spore levels reflects a background of elevated counts in elderly patients as reported previously (6, 7; Stringer and Gilbert, J. Appl. Bacteriol., 1981).

Throughout the series of enteroitis outbreaks, ward D was most heavily involved (Table 1). Certain individuals were cyclically involved and showed recurrence of symptoms several times during the episodes. Acceptable sanitary conditions on this and other wards were difficult to maintain, particularly on weekends with reduced staffing. Many patients had chronic or recurrent fecal incontinence or diarrhea or both. Patient-to-patient transfer was proposed as one possibility, with one individual on ward D suggested as the possible major source. Although to our knowledge such a transfer mechanism has not been reported previously, the lack of evidence relating enteroitis episodes to food sources lends support to such an alternative. The following factors, however, tend to refute such a mechanism: (i) several serotypes were involved in the recurring outbreaks (Fig. 2), and although certain serotypes appear to be related to certain wards, patients in one ward with enteroitis concurrently did not consistently share a common serotype; and (ii) if patient-to-patient transfer were the mechanism, one would not expect that pattern to be so consistent (weekend occurrence), even though sanitary conditions deteriorated on weekends (certain patients were consistently in a state of poor personal hygiene).

A more traditional explanation for the source of the organism would be consumption of contaminated food. No particular relationship in diet was apparent, however, among enteroitis patients distinct from other patients. Also, the lack of isolation of _C. perfringens_ from foods known to be eaten by subsequent enteroitis cases tends to refute this route. Since food samples were refrigerated for up to 72 h before being tested, perhaps low numbers of _C. perfringens_ or a particularly cold-labile strain(s) was present. Further speculation suggests that such low numbers, while not normally constituting an enteroitis-inducing dose, might have a more profound effect on patients whose age and clinical status could facilitate such a process. Recently, Borriello et al. (1) have reported _C. perfringens_ enterotoxin and high _C. perfringens_ spore levels in patients with apparently antibiotic-associated diarrhea (symptoms were considerably more severe in some cases than might be expected for _C. perfringens_ enteritis).

The actual mechanism of the cyclical gastroenteritis inci-
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Patients may remain unknown. Possible external sources of potentially contaminated foods ("home-cooked" dishes brought in by visitors) were eliminated for a time. Radical upgrading of sanitary conditions was undertaken by hospital staff. (Measures included limiting patient mobility to other wards, rigorous cleaning of beds and handrails, etc.) After these measures had been taken, the incidence of enteritis was dramatically reduced. However, occasional cases continued to appear for several months after the peak.

Although the source of C. perfringens was not identified, one very important conclusion can be drawn from the analysis of this very unusual and complex situation. When such a background of elevated C. perfringens spore counts, multiple serotypes, and chronic and recurring fecal incontinence or diarrhea exists, enterotoxin detection provides the definitive evidence for confirmation of C. perfringens etiology. For this reason, caution should be exercised in interpreting the significance of elevated C. perfringens spore counts and serotyping when gastroenteritis outbreaks occur among elderly patients in chronic care facilities.

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LITERATURE CITED