Emerging and Reemerging Clostridia in Human Diseases

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Background

During the past decade, considerable attention was directed toward the emergence and reemergence of various viral, fungal, parasitic and bacterial infectious diseases as significant threats to public health. (6, 14) Included among the published lists of specific infectious diseases and agents are those caused by several aerobic bacterial pathogens, with no or only limited recognition of new threats posed by anaerobes. (17, 20) Recently, species of the genus *Clostridium* (sporeforming anaerobes) have gained recognition either through a dramatic increase in their incidence, or by a threat of increased incidence in the near future.

Purpose

The object of this brief report is to call attention to new threats posed by *Clostridium difficile, C. perfringens,* and certain other *Clostridium* species, particularly in life-threatening blood stream infections, and invasive infections. Although they are of current interest, the scope of this presentation permits only brief mention of some species (e.g., *C. septicum, C. novyi, C. sordellii* and *C. botulinum*). The important role of microbiologic cultures and immunologic procedures or molecular methods as well as pathologic observations (i.e., histopathology) in the diagnosis of infectious diseases will be emphasized.

Clostridium difficile-associated disease (CDAD): a US epidemic

During the past two years, increased rates of CDAD have occurred in many US, Canadian and European medical facilities. This increase has been associated with the emergence of a new epidemic toxinotype III strain of *C. difficile* (i.e., referred to as REA group BI, pulse field NAP1, or PCR Ribotype 027). As of 1 September 2006, the new epidemic strain has been associated with hospital outbreaks in at least 20 states, and associated with higher mortality and morbidity than noted in the past. As reviewed recently by Gerding, the new strain of *C. difficile* produces a third toxin (binary toxin), has a deletion in a gene that down-regulates the production of toxins A and B, thus resulting in the in-vitro production of 16-20 times more toxin, and it is more resistant to newer fluoroquinones (e.g., gatifloxacin and moxifloxacin). (10) This strain has been associated with fulminant CDAD relatively frequently; common complications have included shock, sepsis, ileus (gut paralysis), toxic megacolon and colonic perforation. Treatment (e.g., with metronidazole or vancomycin) has been more challenging, recurrences have been relatively high (> 30%), and colectomy may be required to save the life of the patient. In hospitals, the elderly are particularly at risk for acquiring the disease and for high mortality. Also, it has been suggested that people receiving proton-pump inhibitor medications may be at higher risk for developing CDAD, and that rates of community acquired CDAD may be increasing. (10)

Laboratory testing for CDAD remains a significant challenge. The most sensitive test available is stool culture for *C. difficile*. The problem of false-positive results due to non-toxigenic strains is dealt with by testing the isolates with a cytotoxin neutralization assay (for toxin B) or an EIA procedure for toxins A and B. A significant problem with culture is that it takes 48-96 hours before results are available. Detection of the "common antigen" (glutamate dehydrogenase) of *C. difficile* can be done rapidly (<1 hr) by latex (originally developed by Kohno and colleagues) or EIA. A recently marketed commercial EIA for the common antigen is nearly as sensitive as stool culture for the organism, but must be combined with toxin testing to verify diagnosis. The tissue culture cytotoxin neutralization assay, although specific, is less sensitive than stool culture for *C. difficile*, requires technical expertise to perform, is costly, and requires 24-48 h for a final result. Several EIA procedures are commercially available which detect toxin A, toxin B or both toxins A and B. These are same day assays, but are the least sensitive means of attempting to make a diagnosis. New, rapid, real time-PCR assays for the toxin genes appear promising, but at this writing, none are commercially available in the US.

Clostridium perfringens intestinal illness

Capable of causing a wide range of histotoxic and intestinal infections (e.g., gas gangrene and food poisoning), this species is a reemerging cause of enteric disease in an array of animals and humans. Virulence of *C. perfringens* is explainable largely by its production of 15 different toxins, though individual

isolates produce only some of these toxins. Thus, based on which of the four major lethal toxins is/are produced, *C. perfringens* can be subdivided into one of five toxinotypes (A-E). (4) Type A isolates, which produce alpha-toxin, but not beta-toxin, epsilon-toxin, or iota-toxin, are the most common cause of *C. perfringens* disease in humans.

Enteritis necroticans (EN). Type C isolates, which produce alpha-toxin and beta-toxin, but not epsilon-toxin, or iota-toxin, cause EN (Pig-Bel), a life-threatening illness characterized by ischemic necrosis of the small bowel. (3) Recognized in Papua New Guinea since 1961, where it has been a significant public health problem in children, EN also occurs in other developing countries. EN occurs rarely in developed countries in both children and adults (13, 16) who are malnourished or have chronic illnesses (e.g., diabetes mellitus or alcoholic liver disease). (12) The pathology of pig-bel has been investigated extensively. In many cases the upper small bowel (e.g., jejunum) is involved focally, but there may be extensive involvement of the entire small bowel and parts of the colon as well. The disease is characterized by an acute, patchy, hemorrhagic, necrotizing, ulcerative, and inflammatory disease of the bowel, often with pseudomembranes and sometimes with gas cysts within the involved areas. (3)

***C. perfringens enterotoxin*.** According to Fisher et al., about 1-5% of *C. perfringens* isolates (mostly type A) produce the *C. perfringens* enterotoxin (CPE). (8) Enterotoxigenic strains have been correlated with *C. perfringens* type A food poisoning, one of the most common causes of foodborne illness in the US. (4) Enterotoxigenic type A *C. perfringens* isolates have also been correlated with antibiotic associated diarrhea (AAD), though far less frequently than *C. difficile* (e.g., CPE detected in ~ 3-8% of fecal samples from patients with AAD). (2, 4, 5) Foodborne isolates of *C. perfringens* most frequently have a chromosomal enterotoxin gene (*cpe*), whereas isolates from patients with AAD usually have a plasmid-borne *cpe* gene. (8)

Beta2 toxin. In 1997, Gibert, et al., identified a new toxin, the "beta2" toxin (CPB2), from a strain of *C. perfringens* isolated from a piglet with necrotic enteritis. (11) Since then, the gene for this new toxin (*cpb2*) has also been found in *C. perfringens* isolates from dogs, horses, and other animals. Recently, we investigated the presence of the *cpb2* gene in *C. perfringens* isolates from humans. Using a multiplex PCR based on the method developed by Garmory et al., (9) *C. perfringens* isolates from human stool samples were analyzed looking for the presence of the alpha, beta, epsilon, and iota-toxin genes, which code for the major toxins that are used to type *C. perfringens*, in addition to the *cpb2* and *cpe* genes. All of the isolates, from patients with *C. perfringens* associated illnesses, as well as from healthy volunteers, were type A. Of the isolates examined (by multiplex PCR) thus far from 161 individuals with gastrointestinal illness (mostly AAD), 30% also had the *cpb2* gene, and 22% carried the *cpe* gene. Only 6% of the isolates studied carried both the *cpb2* and the *cpe* genes. In comparison, all of the 100 isolates from healthy volunteers were type A, and 11% were found to carry the *cpb2* gene. Only one of these also carried the *cpe* gene; another carried *cpe* but not *cpb2*. Since our reports in 2003 and 2004 (18, 19), others reported >75% of AAD isolates of *C. perfringens* tested positive for the *cpb2* gene and that >97% of these isolates produced the CPB2 toxin. (8) In addition, it was reported that the *cpe* gene was carried on the same plasmid as the *cpb2* gene and suggested that CPB2 could be an accessory toxin in *C. perfringens* enterotoxin associated AAD. (8)

<u>*Clostridium* species emerging in blood stream infections</u>

Recently, based on 16S rRNA sequences, phenotypic characteristics and antimicrobial susceptibility, "*Clostridium clostridioforme*" was found to comprise a group of three major species that differ in virulence and resistance to a number of antimicrobial agents despite similar colony and microscopic characteristics. Included in the group are *C. bolteae, C. clostridioforme*, and *C. hathewayi*. (7) In my laboratory during the past five years, *C. clostridioforme* was the second most frequently isolated species of *Clostridium* after *C. perfringens* in clinically significant anaerobic bacteremia. (1) These species are also being isolated relatively frequently from other types of infections (e.g., necrotizing fasciitis, intraabdominal abscess, pelvic abscess, peritonitis, wound infections, and others). Another major pathogenic *Clostridium* seen in blood stream infections is *C. septicum*; this often devastating form of septicemia is also associated with underlying gastrointestinal lesions in the neutropenic host, underlying malignancy (e.g., leukemia-lymphoma or colon cancer) and high mortality. (4)

Clostridium sordellii toxic shock syndrome after medical abortion

In 2005, the US Food and Drug Administration (FDA) issued a public health advisory regarding deaths of four women in the US after medical abortion with mifepristone and intravaginal misoprostol. (15) Two of the cases had clinical evidence of toxic shock and microbiologic evidence of intrauterine infection with *C. sordellii*. A case in 2001 from Canada had similar findings, and *C. sordellii* had been reported previously as a cause of pregnancy-associated toxic shock syndrome. The CDC currently encourages health-care providers to report to CDC any cases of postpartum or postabortion toxic shock syndrome. (15)

Conclusions regarding emerging/reemerging _Clostridium_ species

Clearly, further study of the CPB2 toxin's role in human disease is warranted. In addition to the species and diseases mentioned above, *Clostridium novyi*, *C. botulinum* and other species (not otherwise covered herein), some of which are newly described, have emerged or reemerged recently in different clinical settings posing new threats to public health. More will emerge in the future. It is important for clinical microbiology laboratories to differentiate between these species, for clinicians to become aware of the differences between them and for health care professionals in general to recognize their significance and means of treatment. It is especially important also that new laboratory methods and test strategies be developed and made commercially available that will enable clinical microbiology laboratories to be on the cutting edge in addressing these new threats, both now and in the future.

References

1. **Allen, S., J. Siders, R. Batteiger, J. Reynolds, and D. Blue.** 2006. Increased frequency of anaerobic bacteremia at Clarian Hospitals. Abstracts of the 8th Biennial Congress of the Anaerobe Society of the Americas, Boise, ID.
2. **Allen, S., J. Siders, J. Fill, M. Riddell, J. Boone, and R. Carman.** 1995. *Clostridium perfringens* enterotoxin in feces of patients with antibiotic-associated diarrhea. Abstract 25. Anaerobe Pathogens: IX International Symposium of the Society for Anaerobic Microbiology, Churchill College, Cambridge, UK.
3. **Allen, S. D.** 1997. Pig-bel and other necrotizing disorders of the gut involving *Clostridium perfringens.*, p. 717-724*. In* D. H. Connor, F. W. Chandler, D. A. Schwartz, H. J. Manz, and E. E. Lack (ed.), Pathology of Infectious Diseases. Appleton & Lange, Norwalk, CT.
4. **Allen, S. D., C. L. Emery, and D. M. Lyerly.** 2003. *Clostridium,* Chapter 54, p. 835-856. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, J. H. Jorgensen, and R. H. Yolken (ed.), Manual of Clinical Microbiology, 8th ed, vol. 1. ASM Press, Washington.
5. **Asha, N. J., D. Tompkins, and M. H. Wilcox.** 2006. Comparative analysis of prevalence, risk factors, and molecular epidemiology of antibiotic-associated diarrhea due to *Clostridium difficile*, *Clostridium perfringens*, and *Staphylococcus aureus*. J Clin Microbiol **44:**2785-91.
6. **CDC.** 1994. Addressing emerging infectious disease threats: a prevention strategy for the United States. Executive summary. MMWR Recomm Rep., Centers for Disease Control. **43:**1-18.
7. **Finegold, S. M., Y. Song, C. Liu, D. W. Hecht, P. Summanen, E. Kononen, and S. D. Allen.** 2005. *Clostridium clostridioforme*: a mixture of three clinically important species. Eur J Clin Microbiol Infect Dis **24:**319-24.
8. **Fisher, D. J., K. Miyamoto, B. Harrison, S. Akimoto, M. R. Sarker, and B. A. McClane.** 2005. Association of beta2 toxin production with *Clostridium perfringens* type A human gastrointestinal disease isolates carrying a plasmid enterotoxin gene. Mol Microbiol **56:**747-62.
9. **Garmory, H. S., N. Chanter, N. P. French, D. Bueschel, J. G. Songer, and R. W. Titball.** 2000. Occurrence of *Clostridium perfringens* beta2 toxin amongst animals, determined using genotyping and subtyping PCR assays. Epidemiol Infect **124:**61-7.
10. **Gerding, D. N.** 2006. US epidemic of *Clostridium difficile* disease. Abstract III-F1. The 8th Biennial Congress of the Anaerobe Society of the Americas, Boise, Idaho, USA.
11. **Gibert, M., C. Jolivet-Reynaud, and M. R. Popoff.** 1997. Beta2 toxin, a novel toxin produced by *Clostridium perfringens*. Gene **203:**65-73.
12. **Gui, L., C. Subramony, J. Fratkin, and M. D. Hughson.** 2002. Fatal enteritis necroticans (pigbel) in a diabetic adult. Mod Pathol **15:**66-70.
13. **Li, D. Y., A. O. Scheimann, J. G. Songer, R. E. Person, M. Horwitz, L. Resar, and K. B. Schwarz.** 2004. Enteritis necroticans with recurrent enterocutaneous fistulae caused by *Clostridium perfringens* in a child with cyclic neutropenia. J Pediatr Gastroenterol Nutr **38:**213-5.
14. **Institute of Medicine.** 1992. Emerging Infections: Microbial Threats to Health in the United States. National Academy of Sciences, Washington, D.C.
15. **MMWR.** 2005. *Clostridium sordellii* toxic shock syndrome after medical abortion with mifepristone and intravaginal misoprostol --- United States and Canada, 2001-2005. MMWR Weekly **54(Dispatch);1:**1-2.

16. **Petrillo, T. M., C. M. Beck-Sague, J. G. Songer, C. Abramowsky, J. D. Fortenberry, L. Meacham, A. G. Dean, H. Lee, D. M. Bueschel, and S. R. Nesheim.** 2000. Enteritis necroticans (pigbel) in a diabetic child. N Engl J Med **342:**1250-3.
17. **CDC.** 1994. Addressing emerging infectious disease threats: a prevention strategy for the United States. Executive summary. MMWR Recomm Rep **43:**1-18.
18. **Roskens Dalzell, H., M. Lasbury, C. Lee, and S. Allen.** 2004. Phenotypic/genotypic characterization of *cpb2* positive *Clostridium perfringens* isolates from humans with and without antibiotic-associated diarrhea. Abstracts of the 7th Biennial Congress of the Anaerobe Society of the Americas, Annapolis, MD.
19. **Roskens, H., M. Lasbury, C. Lee, and S. Allen.** 2003. Presence of the ß-2 toxin gene in Clostridium perfringens isolates from humans with antibiotic-associated diarrhea and from healthy volunteers. Abstracts of the 3rd World Congress on Anaerobic Bacteria and Infections, Glasgow, Scotland, UK.
20. **Schwartz, D. A., and D. A. Bryan.** 1996. Infectious disease pathology and emerging infections: are we prepared. Arch Pathol Lab Med **120:**117-124.