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PEDIATRIC INFECTIOUS DISEASES (I BROOK, SECTION EDITOR)

Clostridial Infections in Children: Spectrum and Management

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Abstract Clostridia can cause unique histotoxic syndromes produced by specific toxins (e.g., gas gangrene and food poisoning) as well as non-syndromic infections (e.g., abscess, local infections, and blood born infection). Clostridia can also be recovered from various body sites as part of polymicrobial aerobic–anaerobic infection. These include intra-abdominal (peritonitis and abscess), biliary tract, female genital tract, abscess (rectal area and oropharyngeal), pleuropulmonary, central nervous system, and skin and soft-tissue infections. Clostridia were recovered from children with bacteremia of gastrointestinal origin, necrotizing enterocolitis, and sickle cell disease. They have also been isolated in acute and chronic otitis media, chronic sinusitis and mastoiditis, peritonsillar abscesses, and neonatal conjunctivitis. Early and aggressive surgical debridement, decompression, and drainage of affected tissues are critical to successful outcome of histotoxic infections. Effective antimicrobials include penicillin, clindamycin, chloramphenicol, third-generation cephalosporins, carbapenems, and vancomycin.

Keywords Clostridia · Gas gangrene · Antimicrobials · Antimicrobial resistance

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Introduction

Clostridia are gram-positive spore-forming bacilli that account for up to 10 % anaerobic infections in children [1, 2, 3••, 4•, 5]. Clostridia are part of the phylum Firmicutes and based on 16S rDNA sequence analysis can be divided into 11 homology groups. The majority of the clinically species are part of group 1 [6]. The most frequent Clostridia in clinical infections are *Clostridium perfringens*, *Clostridium septicum*, *Clostridium ramosum*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium histolyticum*, *Clostridium fallax*, *Clostridium bifermentans*, and *Clostridium innocuum*.

Clostridia are members of normal human flora in the intestinal tract and vagina [5], and colonize the gut of other vertebrates and insects, and are ubiquitous in soil. The distribution of clostridia in infections is related to their colonization patterns [6].

Clinically important Clostridium species are categorized into three groups: histotoxic species (*C. perfringens*, *C. ramosum*, *C. novyi*, *C. septicum*, *C. bifermentans*, and *C. sordellii*), enterotoxigenic (*C. perfringens* and *Clostridium difficile*), and neurotoxic (*Clostridium tetani* and *Clostridium botulinum*) [7•]. Clostridial species that cause gas gangrene produce extracellular toxins or possess virulence factors such as enzymes or lysis factors. Clostridia that cause gastrointestinal disease produce enterotoxins.

Clostridia can cause unique histotoxic syndromes generated by their specific toxins (e.g., gas gangrene and food poisoning) as well as non-syndromic infections (e.g., abscess, local infections, and blood born infection). Clostridia can also participate in polymicrobial aerobic–anaerobic infections, [3••, 4•, 5, 6, 7•] intra-abdominal (peritonitis, liver, and splenic abscess) [8–11], biliary tract, female genital tract, abscess (rectal area and oropharyngeal), pleuropulmonary, central nervous system, and skin and soft tissue [3••, 4•, 7•].

Clostridia strains (*C. perfringens*, *Clostridium butyricum*, and *C. difficile*) have been recovered from blood and peritoneal cultures of necrotizing enterocolitis and from infants with sudden death syndrome [12–14]. They were isolated from children with bacteremia of gastrointestinal origin [15] and sickle cell disease [16] and have been recovered in acute [17] and chronic [18•] otitis media, chronic sinusitis and mastoiditis [19•, 20•], peritonsillar abscesses [21], and neonatal conjunctivitis [22].

A survey of the isolation of *Clostridium* spp. illustrated the wide spectrum of infections they cause in children. A total of 113 *Clostridium* spp. were recovered from 107/1543 specimens (7.0 % of all specimens) obtained from 96 children [23]. The isolates comprised 43 (38 %) *Clostridium* spp., 37 (33 %) *C. perfringens*, 13 (12 %) *C. ramosum*, five (4 %) *C. innocuum*, six (5 %) *C. botulinum*, three (3 %) *C. difficile*, two (2 %) *C. butyricum*, and one isolate each of *C. bifermentans*, *Clostridium clostridiiforme*, *Clostridium limosum*, and *Clostridium paraputrificum*. Most isolates were from abscesses (38), peritonitis (26), bacteremia (10), and chronic otitis media (7). Predisposing or underlying conditions were present in 31 (32 %) cases: immunodeficiency (12), malignancy (9), diabetes (7), trauma (7), presence of a foreign body (6), and previous surgery (6). They were the only bacterial isolates in 14 (15 %) cases; 82 (85 %) cases had mixed infection.

Clostridium was often recovered mixed with other aerobic and anaerobic bacteria [23]. The species most commonly isolated with clostridia were anaerobic cocci (57); *Bacteroides* spp. (including *Bacteroides fragilis* group) (50), *Escherichia coli* (22), pigmented *Prevotella* or *Porphyromonas* spp. (18), and *Fusobacterium* spp. (10). Most *Bacteroides* spp. and *E. coli* isolates were from abdominal infections and skin and soft tissue infections adjacent to the rectal area; most pigmented prevotella and porphyromonas were from oropharyngeal, pulmonary, and head and neck sites.

Predisposing Conditions

Most clostridial infections arise from an endogenous colonization site, where the normal mucocutaneous barriers are disrupted from surgery, trauma, a perforated viscus, necrotizing enterocolitis, or chemotherapy-related mucositis [3••]. The presence of devitalized tissue and low oxidation-reduction potential facilitates clostridial growth. Contamination of existing wounds or surgical sites can also get infected by soil organisms, contaminated water or objects, and animal and human feces. Clostridia are found in wounds with foreign body and osteomyelitis of long bones, especially in association with wound contamination after trauma by soil or gut flora [4•, 7•].

Clinical Presentations

Histotoxic Infections

C. perfringens is the commonest isolate from gas gangrene (Table 1) [24, 25]. Other clostridia include *C. bifermentans*, *C. sordellii*, *C. septicum*, *C. novyi*, and *C. histolyticum*. The spectrum of disease produced by *C. perfringens* and other histotoxic clostridia ranges in severity from relatively benign conditions (Welch abscess or anaerobic cellulitis), to infections associated with high mortality such as post abortion uterine infection and clostridial myonecrosis (gas gangrene) [26•]. The last is often polymicrobial occurring after bacteremic spread from the gut to traumatized soft tissue. However, clostridial bacteremia can be associated with infections (see above) [12–14, 27] and also be transient, without clinical consequence or represent a contamination.

The predisposing factors for blood borne infection in adults are often colorectal or hematologic malignancy, inflammatory bowel disease, trauma to the colon or female genital tract, injection drug abuse, and hemodialysis [4•, 7•]. Clostridial endocarditis is uncommon [28].

Clostridium sordellii and *C. septicum* infection has often a rapidly fatal course. *C. septicum* septicemia in children usually is associated with severe neutropenia [29], typhlitis, mucositis, or necrotizing enterocolitis. Sometimes sepsis occurs spontaneously [30]. In these instances, blood borne and soft tissue infection progress rapidly along with gas production [31] and survival is poor. Fatal *C. septicum* septicemia and secondary brain abscess can complicate hemolytic uremic syndrome caused by *E. coli* O157:H7 [32, 33], bowel ischemia, or trauma [34•]. *C. sordellii* infections can follow abortion, childbirth, genital tract surgery, skin or soft tissue trauma, or injectable drug abuse [35]. Lethal and hemorrhage toxins are responsible for typical leukemoid reaction and high mortality [36••].

Gas gangrene usually affects muscles compromised by surgery, trauma, or vascular insufficiency and are contaminated with *C. perfringens* spores, usually from foreign material or a medical device [37]. Wounds can be contaminated by *C. perfringens* spores from the skin, dirt, soil, and clothing especially in the lower trunk [38].

The metabolic requirements of *C. perfringens* for growth are the major factors in the establishment of clostridial myonecrosis. Alpha-toxin (a lecithinase) elaboration by C. *perfringens* induces the severe rapidly progressive myonecrosis [39•]. It rapidly lyses cell membranes causing hemolysis, myofibrillar injury, and vascular permeability. Along with the local infection, the patient is alert, anxious, and apprehensive or apathetic and has fever, tachycardia, and diaphoresis and severe systemic manifestations. Shock and multiorgan failure occurs in most individuals.

Table 1 Clinical presentation of histotoxic clostridial infections

Disease	Comment
Localized skin/soft tissue infection	Polymicrobial infection
	Related to trauma or ischemia
	Frequently remains localized but with extensive necrosis Perirectal abscess, decubitus ulcers
Spreading cellulitis/fasciitis	Usual pathogens: *Clostridium ramosum*, *Clostridium perfringens*, *Clostridium septicum*;
	Compartment syndromes
	Mortality rate 50 %
Myonecrosis (gas gangrene)	Usual pathogens: *C. perfringens*, *C. sordellii* for most cases related to trauma or ischemia
	Effects extremities or abdominal wall
	Treatment requires aggressive surgical debridement
	Often fatal
Disseminated myonecrosis	Usual pathogen: *C. septicum*, *C. sordellii*, or *C. perfringens* associated with severe neutropenia, colorectal or hematologic malignancy, intestinal insult
	Characterized by spreading cellulitis and crepitation
	Often fatal
Suppurative visceral infection	Infection polymicrobial often including *C. perfringens* and *C. ramosum*
	Associated with cholangitis, pancreatic disease, pelvic infections, intra-abdominal abscess, and pulmonary aspiration
Bacteremia/septicemia	Usual pathogen: *C. perfringens* (often a benign course), *C. septicum*, and *C. sordellii* (rapidly progressive, leading to disseminated gas gangrene)

Gas gangrene typically starts 1–4 days (6 h to 21 days) following an injury [38]. Initial symptoms are sudden and persistent pain at the injury site and is accompanying by limb "heaviness". Skin rapidly turns cool, pale, and waxy because of ischemia. This is followed by tense swelling, tenderness, pallor, and a thin hemorrhagic exudate; pallor changes to a bronze or magenta discoloration and hemorrhagic (purplish) bullae emerge. Other features include free air inducing soft tissue crepitation, an offensive sweet odor, and brownish serosanguineous discharge.

The infected muscle is initially edematous and pale and acquires a mottled appearance and greenish black gangrene. Its consistency changes from pasty and mucoid to friable and liquefied.

Initial low fever, tachycardia, myalgia, anxiety, and diaphoresis progress rapidly to hypotension, poor perfusion, disseminated intravascular coagulation, and mental status changes [37, 38]. Left untreated leads to high fever, rigors, shock, renal failure, metabolic acidosis, sepsis, coma, and death.

Food Poisoning

Enterotoxins producing *C. perfringens* (mostly heat-resistant type A) can cause gastroenteritis due to ingestion of contaminated food products (mostly meat products) [40]. This occurs after ingestion of vegetative *C. perfringens* present in foods standing at room temperature [41]. Cooking temperature must exceed 120 °C to kill all spores. Cooking at lower temperatures does not eliminate the spores that convert to vegetative forms causing food poisoning.

Clostridial gastroenteritis is a secretory and inflammatory diarrhea. Detection of the enterotoxin gene *cpe* can identify the causing strains. Similarly to *Shigella* toxin, the enterotoxin reduces glucose absorption and water, sodium, and chloride secretion and strips the villous epithelium.

C. perfringens food poisoning is an acute, self-limiting (<24 h) diarrhea appearing 6 to 24 h following ingestion of contaminated food [41]. It is characterized by painful abdominal cramps watery diarrhea, without blood or mucus. Nausea, vomiting, and fever are rare. It is similar to food poisoning caused by *Bacillus cereus* or other viral-, bacterial-, and toxin-related diarrhea [42]. Diagnosis can be made by recovering large quantities of *C. perfringens* from suspect food ($>10^6$ CFU/g) and patient's stool. Enterotoxin can be detected using a cytopathic toxin neutralization assay, latex agglutination, or enzyme immune assays [43].

Neonatal Necrotizing Enterocolitis

Clostridia have been implicated as pathogens in some infants with necrotizing enterocolitis (NEC). However, their definite role in NEC awaits further confirmation.

Pedersen and colleagues [44] cultured *C. perfringens* from the peritoneal fluids of babies who died of NEC and observed Gram-positive bacilli resembling clostridia in necrotic portions of the gut in six of seven infants. Howard et al. [45] reported an outbreak of non-fatal NEC from *C. butyricum*.

Strum and co-workers [46]. recovered *C. butyricum* from the peritoneal fluid and cerebrospinal fluid of a neonate with NEC. Brook et al. [47] recovered *C. difficile* mixed with *Klebsiella pneumoniae* from the peritoneal fluid and blood of a patient with NEC. Warren et al. [48] "recovered *C. perfringens* from the inflamed peritoneal cavity of two newborns with NEC with severe hemolytic anemia. Novak [49] described red blood cell alteration in four patients with NEC. *Clostridium* spp. were recovered in the blood or peritoneal cavity of three of four patients. These strains elaborated red blood cells altering enzymes also in vitro. Alfa et al. [50] described an outbreak of NEC occurred in six neonates within a 2-month period. "Blood cultures from three of these neonates grew the same strain of what appears to be a novel *Clostridium* spp." de la Cochetiere et al. [51••] detected *C. perfringens* species in the first 2 weeks of life of three infants who later displayed symptoms of NEC.

Other Infections

Clostridia can be involved in various often polymicrobial pediatric infections: arthritis [52•], osteomyelitis [52•], skin and soft tissue (often after trauma or foreign body penetration) [35, 53], panophthalmitis [54], intra-abdominal [55], pulmonary [56], intracranial [57], pelvic infections [58], abscesses [5], and panophthalmitis [31]. Clostridial septicemia usually occur in children with malignancy, aplastic anemia, or immunodeficiency and often follows an ischemic gastrointestinal insult, such as neonatal necrotizing enterocolitis or toxic megacolon [7•].

Clostridial necrotizing enteritis (also called enteritis necroticans or pigbel) is rare in developed countries and causes high mortality following consumption of undercooked pork. Type C enterotoxin producing *C. perfringens* is usually the cause [59].

Neutropenic enterocolitis (typhlitis) is associated with clostridia (mostly *C. septicum*) in those with congenital neutropenia, leukemia, or neutropenia [60]. *C. perfringens* and *C. ramosum* can be isolated in septic abortion and puerperal septicemia [61].

Diagnosis

Gas gangrene is diagnosed on clinical grounds. Typically, myonecrosis and crepitation are present. Synergistic nonclostridial anaerobic myonecrosis usually present with less severe systemic symptoms. Localized clostridial cellulitis usually begins gradually, with less pain or systemic toxicity, and is hard to distinguish from exotoxin A-producing group A streptococcal infection. Severe crush injury is often the predisposing injury in anaerobic cellulitis and myonecrosis [37].

Gram stain can demonstrate gram-positive bacilli, without neutrophils. Recovery of *C. perfringens* from a wound is not diagnostic of gas gangrene, and inability to isolate *C. perfringens* does not exclude its diagnosis [42, 62]. Recovery from a deep tissue site should be tried. Singleplex molecular diagnosis and syndromic multiplex molecular detection methods hold promise for early identification of the organism.

Treatment

Early and aggressive surgical debridement, decompression, and drainage are critical to successful outcome of *histotoxic infections*. Amputation of extremities may be needed. Often, several procedures may be required to remove all necrotic or compromised tissue.

The choice of appropriate empirical therapy is complicated by clostridial increased resistance to several antimicrobials by [63••]. Resistance patterns have been monitored locally and nationally, but susceptibility testing of anaerobes at individual hospitals is rarely performed [64•]. The susceptibility of *Clostridium* spp. recovered from a serious infection should, therefore, be determined especially if its susceptibility patterns are unpredictable

Specific antimicrobials against *Clostridium* spp. should be given, even though the infection's environment is anoxic and acidic and detrimental to their efficacy. Penicillin G should be included in regimens for treatment of rapidly spreading clostridial cellulitis, myonecrosis, or septicemia. Most Clostridia (except for some *C. ramosum*, *C. clostridiforme*, and *C. innocuum*) are susceptible to penicillin. However, increasing resistance of *Clostridium* spp. to penicillin has been noted [65]. Some *Clostridium* spp. may resist penicillin/ampicillin through the production of beta-lactamase(s) but are usually inhibited by beta-lactamase inhibitors [65]. Eflux pump activity related to resistance has also been described in Clostridium [66–68]. With the exception of *C. perfringens*, cephalosporins are relatively less active against most species of Clostridium, including *C. difficile* [69•, 70]. Clindamycin, cefoxitin, and metronidazole are clinically inferior to penicillin, and some *Clostridium* spp. (especially *C. ramosum*, *Clostridium tertium*, and *C. sporogenes*) are resistant.

Macrolides show relatively good activity against *C. perfringens*. There is high clindamycin resistance among some *Clostridium* spp. (especially *C. difficile*) [71].

The drug of choice is penicillin G in a dose of 200,000 to 400,000 U/kg/day given intravenously in divided doses every 4 h. Clindamycin suppresses bacterial toxin synthesis. Among the single and combination antimicrobial treatments, metronidazole alone, clindamycin alone, and clindamycin plus penicillin were the most efficacious in a mouse model. "Although

the survival of mice treated with clindamycin plus penicillin was greater than that of mice treated with clindamycin alone, the difference did not reach statistical significance. In contrast, mice treated with a combination of metronidazole and penicillin demonstrated greater mortality than those treated with metronidazole alone." [72••] Other effective antimicrobials include chloramphenicol, third-generation cephalosporins, carbapenems, and vancomycin. Treatment should be continued until the infection has cleared.

Aggressive supportive care is challenging and complex. Non-controlled data supports the use of hyperbaric oxygen (HBO) for gas gangrene [73]. However, surgical and other procedures when indicated should not be delayed for HBO therapy. HBO minimizes tissue loss and diminishing the extent of debridement. Polyvalent equine antitoxin is utilized without supportive data. Other adjuvant therapies include G-CSF, granulocyte transfusions, and intravenous immunoglobulin [34•].

Survival rate is 75–90 % with early, modern, aggressive treatment and depends on disease's location, extent and promptness of debridement. Mortality is high when surgical treatment is delayed.

C. perfringens food poisoning is self-limiting and medical intervention rarely is required [42]. Oral rehydration with hypotonic fluids usually suffices, although sometimes, especially in infants, intravenous hydration may be required. Antimicrobials are not needed.

Prevention

Risk of histotoxic infections can be reduced by adequate cleaning, debridement, and meticulous care of contaminated wounds. Antimicrobials should be given to those with heavily contaminated wounds, which should be left open to air.

Food poisoning can be prevented by appropriate handling of cooked foods (mainly meats). It should be evenly cooked or reheated at least 120 °C, and if not consumed while hot, should be stored at <5 °C [74]. Meat allowed to stand at room temperature can allow growth of *C. perfringens*. Preformed toxin is destroyed with reheating, however, spores are not affected.

Compliance with Ethics Guidelines

Conflict of Interest The author declares that he has no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Dunkle LM, Brotherton TJ, Feigen RD. Anaerobic infections in children: a prospective study. Pediatrics. 1976;57:311–20.
2. Trimourthi MC, Keen BM, Dajani AS. Anaerobic infections in children: a prospective study. J Clin Microbiol. 1976;3:318–23.
3.•• Brook I. Clostridial infection in children. J Med Microbiol. 1995;42:78–82. **A survey of the isolation of Clostridium spp. from 1543 specimens sent to anaerobic microbiology laboratories revealed 113 isolates from 107 specimens (7.0% of all specimens) from 96 children. The isolates comprised 43 (38%) unidentified Clostridium spp., 37 (33%) C. perfringens, 13 (12%) C. ramosum, five (4%) C. innocuum, six (5%) C. botulinum, three (3%) C. difficile, two (2%) C. butyricum, and one isolate each of C. bifermentans, C. clostridiiforme, C. limosum and C. paraputrificum.**
4.• Finegold SM, George WL (eds) Anaerobic Infections in Humans. New York, Academic Press, 1977. **An extensive and thorough textbook that reviews all case reports and studies of anaerobic infections until 1977**.
5. Hatheway CL. Toxogenic clostridia. Clin Microbiol Rev. 1990;3:66–98.
6. Rosebury T. Microorganisms indigenous to man. New York: McGraw-Hill Book Company; 1966.
7.• Brook I. Anaerobic infections diagnosis and management a textbook. New York: Informa Healthcare USA; 2007. **A textbook devoted to the diagnosis and treatment of all anaerobic infections in newborns and children**.
8. Brook I. Bacterial studies of peritoneal cavity and postoperative surgical wound drainage following perforated appendix in children. Ann Surg. 1980;192:208–12.
9. Brook I. A 12 year study of aerobic and anaerobic bacteria in intra-abdominal and postsurgical abdominal wound infections. Surg Gynecol Obstet. 1989;169:387–92.
10. Brook I, Frazier E. Microbiology of liver and spleen abscesses. J Med Microbiol. 1998;47:1075–80.
11. Brook I, Frazier E. Aerobic and anaerobic microbiology of retroperitoneal abscesses. Clin Infect Dis. 1998;26:938–41.
12. Cashore WJ, Peter G, Lauermann M, Stonestreet BS, Oh W. Clostridium colonization and clostridial toxin in neonatal necrotizing enterocolitis. J Pediatr. 1981;98:308–11.
13. Sturm R, Staneck JL, Stauffer LR, Neblett 3rd WW. Neonatal necrotizing enterocolitis associated with penicillin resistant *Clostridium butyricum*. Pediatrics. 1980;66:928–31.
14. Cooperstock MS, Steffen E, Yolken R, Onderdonk A. *Clostridium difficile* in normal infants and sudden infant death syndrome: an association with infant formula feeding. Pediatrics. 1982;70:91–5.
15. Brook I, Controni G, Rodriguez W, Martin WJ. Anaerobic bacteremia in children. Am J Dis Child. 1980;134:1052–6.
16. Brook I, Gluck RS. *Clostridium paraputrificum* sepsis in sickle cell disease: a report of a case. South Med J. 1980;73:1644–5.
17. Brook I, Schwartz RH, Controni G. *Clostridium ramosum* isolation in acute otitis media. Clin Pediatr. 1979;18:699–70.
18.• Brook I. Microbiology of chronic otitis media with perforation in children. Am J Dis Child. 1980;130:564–66. **The middle ear aspirate and a swab specimen of the external auditory canal were cultured aerobically and anaerobically from 68 children with chronic otitis media (COM). Seventy-eight isolates were recovered from the middle ears, 99 from the external ear canals, and**

95 from both sites. Aerobes only were isolated from 33 patients (48.5 %), nine (13.2 %) had only anaerobes, and 26 (38 %) had both aerobes and anaerobes. There were 99 aerobic isolates. Aerobes recovered icluded Pseudomonas aeruginosa, Staphylococcus aureus, Proteus spp., Klebsiella pneumoniae, and Haemophilus influenzae. There were 74 anaerobes isolated. Anaerobes included were anaerobic Gram-positive cocci, Bacteroides spp., and Clostridium spp. These findings demonstrate the polymicrobial bacteriology of COM in children. Cultures collected from the external auditory canals prior to their sterilization can be misleading.

19.• Brook I. Bacteriological features of chronic sinusitis in children. JAMA. 1981;246:967–69. **Aspiration of chronically inflamed sinuses was performed in 40 children. The sinuses were the maxillary (15 cases), ethmoid (13), and frontal (seven). Pansinusitis was present in five patients. A total of 121 isolates (97 anaerobic and 24 aerobic) were recovered from 37 sinuses, Anaerobes were recovered from all specimens, and in 14 cases (38 %) mixed with aerobic organisms. The predominant anaerobes were Bacteroides spp., anaerobic Gram-positive cocci, and Fusobacterium spp. The predominant aerobes were Staphylococcus aureus, and Haemophilus spp. These findings illustrate the major role of anaerobes in chronic sinusitis in children**.

20.• Brook I. Aerobic and anaerobic bacteriology of chronic mastoiditis in children. Am J Dis Child. 1981;135:478–9. **Aspiration of chronically inflamed mastoids was performed in 28 children. A total of 90 isolates (61 anaerobic and 29 aerobic) were recovered from all mastoids sinuses, Anaerobes were recovered from 4 (17) specimens, aerobes only from one (4 %), and mixed anaerobes with aerobes in 19 (79 %). The predominant anaerobes were anaerobic gram negative bacilli, anaerobic Gram-positive cocci, and Clostridium, Actinomyces spp., and Fusobacterium spp. The predominant aerobes were Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae. These findings illustrate the polymicrobial aerobic and anaerobic nature of chronic mastoiditis in children**.

21. Brook I. Aerobic and anaerobic bacteriology of peritonsillar abscess in children. Acta Pediatr Scand. 1981;70:831–5.

22. Brook I, Martin WJ, Finegold SM. Effect of silver nitrate application on the conjunctival flora of the newborn and the occurrence of clostridial conjunctivitis. J Pediatr Ophthalmol Strabismus. 1978;15:173–83.

23. Brook I. Recovery of anaerobic bacteria from clinical specimens in 12 years at two military hospitals. J Clin Microbiol. 1988;26:1181–8.

24. Brook I. Facial gas gangrene after penetrating injury. Pediatr Infect Dis J. 1990;9:222–3.

25. Brook I. Microbiology and management of infectious gangrene in children. J Pediatr Orthop. 2004;24:587–92.

26.• Brook I. Microbiology of necrotizing fasciitis associated with omphalitis in the newborn infant. J Perinatol. 1998;18:28–30. **Thirty eight bacterial isolates (21 aerobic and facultative and 17 anaerobic) were recovered from 11 newborn infants with periumbilical necrotizing fasciitis. Aerobic or facultative bacteria only were present in 1 specimen (9 %), anaerobes only in 2 (18 %), and mixed aerobic and anaerobic flora in 8 (73 %). The predominant isolates were Peptostreptococcus spp., Bacteroides fragilis group; Clostridium spp., streptococcus group B; and Staphylococcus aureus, streptococcus group D, Escherichia coli, and Proteus mirabilis**.

27. Caya JG, Truant AL. Clostridial bacteremia during the first year of life: an analysis of 53 patients including two new cases. Anaerobe. 2000;6:1–9.

28. Brook I. Infective endocarditis caused by anaerobic bacteria. Arch Cardiovasc Dis. 2008;101:665–76.

29. Barnes C, Gerstle JT, Freedman MH, et al. *Clostridium septicum* myonecrosis in congenital neutropenia. Pediatrics. 2004;114:e757–60.

30. Bratton SL, Krane EJ, Park JR, et al. *Clostridium septicum* infections in children. Pediatr Infect Dis J. 1992;11:569–75.

31. Pinzon-Guzman C, Bashir D, McSherry G, Beck MJ, Rocourt DV. *Clostridium septicum* gas gangrene in a previously healthy 8-year-old female with survival. J Pediatr Surg. 2013;48:e5–8.

32. Hunley TE, Spring MD, Peters TR, et al. *Clostridium septicum* myonecrosis complicating diarrhea-associated hemolytic uremic syndrome. Pediatr Nephrol. 2008;23:1171–5.

33. Williams EJ, Mitchell P, Mitra D, Clark JE. A microbiological hazard of rural living: *Clostridium septicum* brain abscess in a child with *E coli* 0157 associated haemolytic uraemic syndrome. BMJ Case Rep. 2012 Jun 25;2012 10.1136/bcr-2012-00642

34.• Smith-Slatas CL, Bourque M, Salazar JC. *Clostridium septicum* infections in children: a case report and review of the literature. Pediatrics. 2006;117:e796–805. **A child who survived a severe case of C septicum myonecrosis involving both abdominal and thoracic cavities is presented. Forty seven cases of C septicum infection; of these, 22 (47 %) were cases of C septicum associated with myonecrosis were reviewed. Conditions related with C septicum infection in children were of 3 categories: patients with neutrophil dysfunction; patients with associated bowel ischemia; and patients with a history of trauma. Malignancies were found in 49% of the cases, cyclic or congenital neutropenia in 21 %, hemolytic-uremic syndrome in 11 %, structural bowel ischemia in 4 %, and local extremity trauma in 6 %. In addition, 6 % of the cases had no known underlying disorder**.

35. Brook I. Management of human and animal bite wounds: an overview. Adv Skin Wound Care. 2005;18:197–203.

36.•• Vidor C, Awad M, Lyras D. Antibiotic resistance, virulence factors and genetics of *Clostridium sordellii*. Res Microbiol. 2015;166:368–74. **This review will summarise studies involving the genetics of C. sordellii, including an antibiotic resistance profile, the genetic determinants of virulence and mutagenesis of C. sordellii.**

37. Jamal N, Teach SJ. Necrotizing fasciitis. Pediatr Emerg Care. 2011;27:1195–9.

38. Brook I. Microbiology and management of soft tissue and muscle infections. Int J Surg. 2008;6:328–38.

39.• Uzal FA, Freedman JC, Shrestha A, et al. Towards an understanding of the role of *Clostridium perfringens* toxins in human and animal disease. Future Microbiol. 2014;9:361–77. **Clostridium perfringens >16 toxins that cause histotoxic and intestinal infections in humans and animals were reviewed. C. perfringens uses chromosomally encoded alpha toxin (a phospholipase C) and perfringolysin O (a pore-forming toxin) during histotoxic infections. In contrast, this bacterium causes intestinal disease by employing toxins encoded by mobile genetic elements, including C. perfringens enterotoxin, necrotic enteritis toxin B-like, epsilon toxin and beta toxin. The intestinal disease-associated toxins vary in their target specificity, when they are produced (sporulation vs vegetative growth), and in their sensitivity to intestinal proteases. Producing many toxins with diverse characteristics likely imparts virulence flexibility to C. perfringens so it can cause an array of diseases**.

40. Grass JE1, Gould LH, Mahon BE. Epidemiology of foodborne disease outbreaks caused by *Clostridium perfringens*, United States, 1998–2010. Foodborne Pathog Dis. 2013;10:131–6.

41. Bennett SD, Walsh KA, Gould LH. Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens* and *Staphylococcus aureus*—United States, 1998–2008. Clin Infect Dis. 2013;57:425–33.

42. Grimwood K, Forbes DA. Acute and persistent diarrhea. Pediatr Clin North Am. 2009;56:1343–61.

43. Miyamoto K, Li J, McClane BA. Enterotoxigenic *Clostridium perfringens*: detection and identification. Microbes Environ. 2012;27:343–9.
44. Pedersen PV, Hansen FH, Halveg AB, Christiansen ED. Necrotizing enterocolitis of the newborn—is it gas-gangrene of the bowel? Lancet. 1976;2:715–6.
45. Howard FM, Flynn DM, Bradley JM, Noone P, Szawatkowski M. Outbreak of necrotising enterocolitis caused by *Clostridium butyricum*. Lancet. 1977;2:1099–102.
46. Sturm R, Staneck JL, Stauffer LR, Neblett 3rd WW. Neonatal necrotizing enterocolitis associated with penicillin-resistant, toxigenic *Clostridium butyricum*. Pediatrics. 1980;66:928–31.
47. Brook I, Avery G, Glasgow A. *Clostridium difficile* in pediatric infection. J Infection. 1982;4:253–7.
48. Warren S, Schreiber JR, Epstein MF. Necrotizing enterocolitis and hemolysis associated with *Clostridium perfringens*. Am J Dis Child. 1984;138:686–8.
49. Novak RW, Klein RL, Novak PE. Necrotizing enterocolitis, hemolysis, and *Clostridium perfringens*. Am J Dis Child. 1985;139:114–5.
50. Alfa MJ, Robson D, Davi M, Bernard K, Van Caeseele P, Harding GK. An outbreak of necrotizing enterocolitis associated with a novel clostridium species in a neonatal intensive care unit. Clin Infect Dis. 2002;35:S101–5.
51.•• de la Cochetiere MF, Piloquet H, des Robert C, Darmaun D, Galmiche JP, Roze JC. Early intestinal bacterial colonization and necrotizing enterocolitis in premature infants: the putative role of clostridium. Pediatr Res. 2004;56:366–70. **The study explore the putative relationship between pathogen microorganisms and NEC, by conducted a prospective case–control study in 12 preterm infants with a new approach based on molecular techniques. It demonstrates that the use of molecular techniques based on the study of bacterial 16S rRNA genes allowed the recognition of C. perfringens species in three infants who later displayed symptoms of NEC.**
52.• Brook I. Microbiology and management of joint and bone infections due to anaerobic bacteria. J Orthop Sci. 2008;13:160–9. **The review describes the microbiology, diagnosis, and management of septic arthritis and osteomyelitis due to anaerobic bacteria. The predominant anaerobes in arthritis are anaerobic Gram-negative bacilli (AGNB), Fusobacterium spp., Peptostreptococcus spp., Clostridium spp., and Propionibacterium acnes. Infection with P. acnes is associated with a prosthetic joint, previous surgery, and trauma. B. fragilis group is associated with distant infection, Clostridium spp. with trauma, and Fusobacterium spp. with oropharyngeal infection. The predominant anaerobes in osteomyelitis are Bacteroides, Peptostreptococcus, Fusobacterium, Clostridium spp. and P. acnes. Conditions predisposing to bone infections are vascular disease, bites, contiguous infection, peripheral neuropathy, hematogenous spread, and trauma. Pigmented Prevotella and Porphyromonas spp. are mostly isolated in skull and bite infections, members of the B. fragilis group in hand and feet infections, and Fusobacterium spp. in skull, bite, and hematogenous long bone infections**.
53. Brook I. Aerobic and anaerobic microbiology of necrotizing fasciitis in children. Pediatr Dermatol. 1996;13:281–4.
54. Levitt JM, Stam J. *Clostridium perfringens* panophthalmitis. Arch Ophthalmol. 1970;84:227–8.
55. Brook I. Microbiology and management of intra-abdominal infections in children. Pediatr Int. 2003;45:123–9.
56. Brook I. Anaerobic pulmonary infections in children. Pediatr Emerg Care. 2004;20:636–40.
57. Brook I. Brain abscess in children: microbiology and management. J Child Neurol. 1995;10:283–8.
58. Brook I. Microbiology and management of polymicrobial female genital tract infections in adolescents. J Pediatr Adolesc Gynecol. 2002;15:217–26.
59. Songer JG. Clostridia as agents of zoonotic disease. Vet Microbiol. 2010;140:399–404.
60. van de Wetering MD, Kuijpers TW, Taminiau JA, et al. Pseudomembranous and neutropenic enterocolitis in pediatric oncology patients. Support Care Cancer. 2003;11:581–6.
61. Meites E, Zane S, Gould C. Fatal *Clostridium sordellii* infections after medical abortions. N Engl J Med. 2010;363:1382–3.
62. Temple AM, Thomas NJ. Gas gangrene secondary to *Clostridium perfringens* in pediatric oncology patients. Pediatr Emerg Care. 2004;20:457–9.
63.•• Brook I, Wexler HM, Goldstein EJ. Antianaerobic antimicrobials: spectrum and susceptibility testing. Clin Microbiol Rev. 2013;26:526–46. **The spectrum of efficacy, antimicrobial resistance mechanisms, and resistance patterns against anaerobic bacteria are described**.
64.• Goldstein EJC, Citron DM, Goldman PJ, Goldman RJ. National survey of anaerobic culture and susceptibility methods: III. Anaerobe. 2008;14:68–72. **The study assessed the status of anaerobic bacteriology in the US, in 150 hospitals. The antimicrobials most often tested in hospital labs were penicillin/ampicillin and clindamycin, metronidazole and cefotetan and ampicillin/sulbactam, piperacillin/tazobactam, cefoxitin, imipenem, and chloramphenicol. The survey suggested that while many labs are processing anaerobic cultures, especially blood cultures, the identification of isolates and the performance of antimicrobial susceptibility testing of isolates are in disarray and in great need of improvement**.
65. Appelbaum PC, Spangler SK, Pankuch GA, Philippon A, Jacobs MR, Shiman R, et al. Characterization of a beta-lactamase from clostridium clostridioforme. J Antimicrob Chemother. 1994;33:33–40.
66. Rafii F, Park M, Carman RJ. Characterization of an ATP-binding cassette from *Clostridium perfringens* with homology to an ABC transporter from clostridium hathewayi. Anaerobe. 2009;15:116–21.
67. Rafii F, Park M, Wynne R. Evidence for active drug efflux in fluoroquinolone resistance in *Clostridium hathewayi*. Chemotherapy. 2005;51:256–62.
68. Spigaglia P, Barbanti F, Mastrantonio P. Multidrug resistance in European *Clostridium difficile* clinical isolates. J Antimicrob Chemother. 2011;66:2227–34.
69.• Hecht DW. Prevalence of antibiotic resistance in anaerobic bacteria: worrisome developments. Clin Infect Dis. 2004;39:92–7. **The review describes how antibiotic-resistant anaerobic bacteria have become increasingly recognized as a confounding factor in the selection of therapeutic agents. It highlights how the use of potent, broadspectrum antibiotics as empirical therapy, along with appropriate adjunctive measures, has masked the magnitude of the antibiotic resistance problem that parallels that observed for non-anaerobic pathogens. It concludes that the use of standardized testing methods that recognize resistance and an understanding of resistance mechanisms have become essential for the treatment of patients and the development of new agents**.
70. Goldstein EJC, Citron DM, Cole RE, Rangel DM, Seid AS, Ostovari MI. Cefoxitin in the treatment of aerobic/anaerobic infections: prospective correlation of in vitro susceptibility methods with clinical outcome. Hospital Prac Symp Suppl. 1990;25 Suppl 4:38–45.
71. Aldridge K, Aldridge KE, Ashcraft D, Cambre K, Pierson CL, Jenkins SG, et al. Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of *Bacteroides fragilis* group, *Prevotella*, *Fusobacterium*, *Porphyromonas*, and

Peptostreptococcus species. Antimicrob Agents Chemother. 2001;45:1238–43.

72.•• Stevens DL, Laine BM, Mitten JE. Comparison of single and combination antimicrobial agents for prevention of experimental gas gangrene caused by *Clostridium perfringens*. Antimicrob Agents Chemother. 1987;31:213–8. **The efficacy of antimicrobial therapy of experimental C. perfringens infection in mice showed that combination antimicrobial therapy did not improve survival compared to that achieved with metronidazole or clindamycin alone, and some combinations significantly reduced survival.**

73. Kaide CG, Khandelwal S. Hyperbaric oxygen: applications in infectious disease. Emerg Med Clin North Am. 2008;26: 571–95.

74. Hillers VN, Medeiros L, Kendall P, et al. Consumer food-handling behaviors associated with prevention of 13 foodborne illnesses. J Food Prot. 2003;66:1893–9.