CLINICAL INFECTIONS

Clostridial infection in children

I. BROOK

Department of Pediatrics, Georgetown University School of Medicine, Washington DC, USA

Summary. 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*. Most clostridial isolates were from abscesses (38), peritonitis (26), bacteraemia (10), and chronic otitis media (7). Predisposing or underlying conditions were present in 31 (32%) cases. These were immunodeficiency (12), malignancy (9), diabetes (7), trauma (7), presence of a foreign body (6) and previous surgery (6). The clostridia were the only bacterial isolates in 14 (15%) cases; 82 (85%) cases had mixed infection. The species most commonly isolated with clostridia were anaerobic cocci (57), *Bacteroides* spp. (*B. fragilis* group) (50), *Escherichia coli* (22), pigmented *Prevotella* or *Porphyromonas* spp. (18) and *Fusobacterium* spp. (10). Most *Bacteroides* and *Escherichia coli* isolates with clostridia were from abdominal infections and skin and soft tissue infections adjacent to the rectal area; most pigmented *Prevotella* and *Porphyromonas* isolates were from oropharyngeal, pulmonary, and head and neck sites. Antimicrobial therapy was given to all patients, in conjunction with surgical drainage in 34 (35%). Only two patients died. These data illustrate the importance of *Clostridium* spp. in paediatric infections.

Introduction

Recognition of the role of anaerobic bacteria in paediatric infections has increased recently.¹ However, most of the interest has focused on *Bacteroides* spp.; other groups of organisms such as *Clostridium* spp. have not been studied as adequately. This article summarises experience of the isolation of clostridial species from infections in children over a period of 17 years. Some of the data were published previously in separate articles describing the role of anaerobic bacteria in paediatric infections,¹ but the role of the clostridia was not highlighted in those studies, as they were isolated from only a small proportion (< 10%) of the patients. This review also includes cases not previously presented that were seen after those studies were completed.

Patients and methods

Patients

The children included in the study were investigated between June 1973 and June 1990 at the following hospitals: University of California Medical Center, Los Angeles County Medical Center and Serra Memorial Hospital in Los Angeles, CA; Children's Hospital National Medical Center and South-East Medical Center in Washington, DC; and National Naval Medical Center in Bethesda, MD. Records from the clinical microbiology laboratories were reviewed to identify patients with clostridial infections. The case histories of all patients from whom *Clostridium* spp. had been isolated were reviewed to ascertain the presence and site of infection, associated micro-organisms, disease process, and possible predisposing or associated conditions.

Microbiological investigations

Blood was collected aseptically from patients suspected of having bacteraemia and was inoculated (10% v/v) into one bottle each of two commercially

Received 21 March 1994; revised version received 30 July 1994; accepted 3 Aug. 1994.

Correspondence should be sent to Dr I. Brook, PO Box 70412, Chevy Chase, MD 20813-0412, USA.

obtained broth media, both under vacuum and with CO₂. For other sites, the microbiology laboratories accepted only specimens that were collected properly by a method that avoided contamination by the normal skin or mucosal flora,² and submitted in transport media appropriate for anaerobic bacteria. These were specimens obtained during surgery, aseptic aspiration of body fluids, or needle or biopsy aspiration of abscesses or body cavities. Aspirates of lungs were obtained through transtracheal aspiration or biopsy. Urine was collected through suprapubic aspiration. When possible, pus and fluid samples were collected and transported to the laboratory in syringes within 30 min. Most specimens, except blood, were submitted in Port-A-Cul transport swab or liquid systems (BBL, Cockeysville, MD, USA). However, exact records of the transport media used were not available in all instances.

The specimens were inoculated on to pre-reduced vitamin K₁-enriched *Brucella* blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin, an anaerobic blood agar plate containing colistin sulphate and naladixic acid, and an enriched thioglycolate broth (containing haemin, sodium carbonate and vitamin K₁).² Cultures were incubated in GasPak jars (BBL) and examined after 48 and 96 h. Plates that showed growth were held until the organisms were processed and identified. All cultures that showed no growth were incubated for at least 5 days. Organisms were identified by the API Anaerobic System (Analytab Products, Plainview, NY, USA) or the Minitek System (BBL). When complete identification was not possible by these methods, other carbohydrate tests (Scott Laboratories, Fiskeville, RI, USA) and gas-liquid chromatography^{2,3} were performed as needed to identify the organisms. The criteria for identification were according to previously published guidelines.^{2,3}

Results

From the 1543 specimens examined for anaerobic bacteria, 113 clostridia were isolated from 107 specimens (7.0% of all specimens examined) from 96 patients. 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 (1%) of *C. bifermentans*, *C. clostridiiforme*, *C. limosum* and *C. paraputrificum* (tables I and II). Clinical records were not available for another six isolates from five patients. These cases were not included in the study.

Most infections were polymicrobial—in 82 (85%) patients—but in 14 (15%) patients a *Clostridium* sp. was isolated in pure culture. Most clostridia were isolated from abscesses (38 isolates; 34%), peritonitis (26; 23%), bacteraemia (10; 9%), including one neonatal bacteraemia, and chronic otitis media

Table I. *Clostridium* spp. isolated from 96 children

Type of infection	Number of specimens		Number of isolates										
	Total	With <i>Clostridium</i> sp. (as sole isolate)	<i>C. bifermentans</i>	<i>C. botulinum</i>	<i>C. butyricum</i>	<i>C. clostridiiforme</i>	<i>C. difficile</i>	<i>C. innocuum</i>	<i>C. limosum</i>	<i>C. paraputrificum</i>	<i>C. perfringens</i>	<i>C. ramosum</i>	<i>Clostridium</i> spp. Total
Neonatal infection													
bacteraemia	121	1 (1)				1							1
cholangitis	8	3 (1)											3
conjunctivitis	35	2 (1)											2
omphalitis	23	2	1										2
scalp	23	1											1
Older children													
abscesses	384	38 (1)						1			16	3	21
bacteraemia	42	9 (7)		2			1				4	1	9
burn wound	180	1				1							1
decubitus ulcer	58	2									1		2
ear: acute otitis	28	3 (1)											3
chronic otitis	94	7 (1)							1				5
mastoiditis	24	3										1	2
lungs: empyema	72	1									1		1
pneumonia	74	1											1
osteomyelitis	26	4 (1)									2	1	4
peritonitis	116	26		4	2		1	4			6	5	29
sinusitis	45	3											3
Total	1543	107 (14)	1	6	2	1	3	5	1	1	37	13	43

Table II. *Clostridium* sp. isolated from 38 abscesses in children

Type of abscess	Total number of abscesses	Number of specimens with <i>Clostridium</i> sp.	Number of isolates of			
			<i>C. innocuum</i>	<i>C. ramosum</i>	<i>C. perfringens</i>	<i>Clostridium</i> sp.
Abdominal	38	12	1	2	5	7
Intracranial	40	1			1	
Liver	8	1			1	
Perinephric	6	1			1	
Perirectal	28	3			2	
Peritonsillar	16	3				3
Pilonidal	25	4			2	2
Retropharyngeal	14	2				2
Skin and soft tissue	195	6		1	2	3
Subphrenic	14	5			2	4
Total	384	38	1	3	16	21

(7; 6%). Predisposing or underlying conditions were noted in 31 (32%) cases. A single condition was noted in 18 (19%) instances, two such conditions in 10 (10%) and three conditions in three (3%). These were immunodeficiency (12; 13%), malignancy (9; 10%), diabetes (7; 8%), trauma (7; 8%), presence of a foreign body (6; 6%) and previous surgery (6; 6%).

There were 240 other isolates mixed with *Clostridium* spp. in the 93 specimens from mixed infections; 168 (70%) were anaerobes and 72 (30%) were aerobes or facultative species. The number of isolates varied from two to seven (average 2.6/specimen, 1.8 anaerobic and 0.8 aerobic or facultative). The organisms isolated most commonly with clostridia were anaerobic cocci (57), *Bacteroides* spp. (50), pigmented *Prevotella* and *Porphyromonas* spp. (18), *Fusobacterium* spp. (10), *Escherichia coli* (22), non-haemolytic streptococci (10) and α -haemolytic streptococci (7). Most *Bacteroides* and *E. coli* isolates were cultured with clostridia from intra-abdominal and peri-rectal skin and soft tissue infections, whereas most pigmented *Prevotella* and *Prophyromonas* isolates were from oropharyngeal, pulmonary and head and neck sites.

Of the 79 patients with mixed infection, six were neonates (table I). One had conjunctivitis in which *C. bifermentans* was mixed with a *Peptostreptococcus* sp. Similar organisms were also isolated from the mother's cervix immediately after delivery. The patient responded to therapy with penicillin eye drops. Two neonates had omphalitis; one was born to a mother with premature rupture of membranes and amnionitis. Two had ascending cholangitis following hepatic portoenterostomy for biliary atresia, and one had a scalp abscess as a result of fetal monitoring; the latter patient also had bacteraemia due to *B. fragilis*.

Of the 73 other patients with mixed infections, 37 had abscesses (table II), of which the most common were abdominal (12), rectal area (7), skin and soft tissue (6), subphrenic (5), peritonsillar (3) and retropharyngeal (2). The other organisms isolated most commonly with clostridia from abscesses were *Peptostreptococcus* spp. (25 isolates), *Bacteroides* spp. (19),

E. coli (17), pigmented *Prevotella* and *Porphyromonas* spp. (7) and *Staphylococcus aureus* (5). *Peptostreptococci* were isolated mostly from abdominal, oro-facial, and head and neck abscesses; *B. fragilis* and *E. coli* were isolated predominantly from abdominal and peri-rectal abscesses.

Amongst the patients with mixed infection of the ear (table I), two had acute and six had chronic otitis media and three had chronic mastoiditis. The most common organisms accompanying the clostridia were *Peptostreptococcus* spp., *Bacteroides* spp. and gram-negative aerobic or facultative bacteria. Cholesteretoma was present in three of the patients with chronic otitis media and in two with chronic mastoiditis. The three clostridial isolates from chronic sinusitis were mixed with oral organisms. There was only a single *Clostridium* isolate (*C. clostridiiforme*) from 180 patients with burn wounds; it was isolated in mixed culture with *B. fragilis* from a burn of the thigh. In specimens from the respiratory tract, the single clostridial isolate from aspiration pneumonia was mixed with *Klebsiella pneumoniae* in a child who suffered from mental retardation and the one patient with a clostridial isolate from empyema had an associated lung abscess (which was not cultured). There were 26 clostridial isolates from cases of peritonitis; 19 were from children with ruptured appendix, five with abdominal trauma, and two with spontaneous peritonitis. The clostridia were mostly mixed with *Peptostreptococcus* spp., *Bacteroides* spp. and enterobacteria. Of the three osteomyelitis isolates, two were from temporal bone infections, one associated with chronic otitis media and one with chronic mastoiditis, and one was from an infected coccyx associated with a decubitus ulcer. The two isolates from this and another decubitus ulcer were from the buttocks area. Organisms of intestinal origin were mixed with these clostridia. There were two cases of polymicrobial bacteraemia, one in a 5-year-old child with a rectal abscess and leukaemia, and the other with abdominal trauma and ruptured viscus.

Clinical and microbiological data on the 14 patients from whom clostridia were isolated in pure culture are

Table III. Summary of 14 patients infected with a *Clostridium* sp. as sole isolate

Case no.	Age	Sex	Diagnosis	Culture site	<i>Clostridium</i> sp. isolated	Therapy	Outcome
1	2 d	F	Conjunctivitis	Conjunctival sac	<i>C. perfringens</i>	Penicillin 2% eye drops	Cured
2	3 m	M	Cholangitis; following porto-enterostomy	Bile duct	<i>C. perfringens</i>	Penicillin + gentamicin	Cured
3	1 m	M	Necrotising enterocolitis	Blood, peritoneal cavity	<i>C. difficile</i>	Penicillin + gentamicin	Died
4	1 y 4 m	M	Bacteraemia: necrotising enterocolitis	Blood, peritoneal cavity	<i>C. difficile</i>	Ampicillin + gentamicin	Died
5	10 y	F	Sepsis; sickle cell disease	Blood	<i>C. paraputrificum</i>	Penicillin	Cured
6	13 y	M	Sepsis; sickle cell disease	Blood, peritoneal cavity	<i>C. perfringens</i>	Penicillin + gentamicin	Cured
7	3 y 6 m	F	Sepsis; perforated viscus	Blood	<i>C. ramosum</i>	Penicillin	Cured
8	7 y 6 m	M	Sepsis; diarrhoea	Blood	<i>C. perfringens</i>	Penicillin	Cured
9	15 y	F	Sepsis	Blood	<i>C. perfringens</i>	Penicillin	Cured
10	10 y	F	Sepsis; leukaemia	Blood	<i>C. perfringens</i>	Penicillin	Died
11	13 y	M	Post-traumatic osteomyelitis	Left femur	<i>C. perfringens</i>	Penicillin	Cured
12	11 y	F	Rectal abscess; leukaemia	Abscess cavity	<i>C. perfringens</i>	Amoxycillin	Cured
13	3 y	M	Chronic otitis media	Middle ear	<i>C. limosum</i>	Amoxycillin	Cured
14	3 y 6 m	M	Acute otitis media	Middle ear	<i>C. ramosum</i>	Amoxycillin	Cured

listed in table III. These included eight children with sepsis or bacteraemia (including one neonate) (patients nos. 3–10), two with otitis (one acute and one chronic) and one case each of neonatal conjunctivitis, cholangitis, osteomyelitis, and peri-rectal abscess. Two patients with *C. perfringens* infection died (2%); one was a neonate (patient no. 4) with necrotising enterocolitis, and the other (no. 10) had lymphocytic leukaemia.

Antimicrobial therapy was administered to all patients and combined with surgical drainage or correction of pathological abnormalities in 34 (35%). The antimicrobial agents administered were penicillin in 43, an aminoglycoside in 31, clindamycin in 28, a cephalosporin in 19, metronidazole in 15, vancomycin in five and erythromycin in two.

Discussion

This review confirms that clostridia are commonly isolated from various infections in children. They are especially prevalent in abscesses (mostly abdominal, rectal area and oropharyngeal), peritonitis and otolaryngological infections, as has been shown in adults.⁴ The distribution of clostridia in these infections is explained by their prevalence in the normal gastrointestinal and cervical flora from where they may originate.⁵

The clostridia found most frequently in clinical infections are *C. perfringens*, *C. ramosum*, *C. botulinum* and *C. innocuum*.⁴ *C. perfringens* is a member of the intestinal flora of man and animals, is found in soil and is the most frequently encountered histotoxic clostridial species. It elaborates several necrotising extracellular toxins and is easily isolated and identified in the clinical laboratory. *C. perfringens* seldom produces spores *in vivo*. It can be recognised in direct smears of

a purulent exudate by the presence of stout gram-variable rods of varying length, frequently surrounded by a capsule, and can cause a devastating illness with high mortality.^{1,4} Clostridial bacteraemia is associated with extensive tissue necrosis, haemolytic anaemia and renal failure.⁴ *C. perfringens* accounted for 48% of all clostridial isolates in adults and children in a recent survey in two military hospitals,⁶ and was isolated primarily from wounds (26% of isolates), blood (16%), abdomen (14%), and obstetrical and gynaecological infections (13%). *C. butyricum* is isolated mostly from infections of the abdomen, abscesses, bile, wounds and blood.^{1,4} It was reported⁷ from blood cultures from 12 neonates with necrotising enterocolitis in one study, but the association of this species with the disease has not been established. Strains of *C. perfringens*, *C. butyricum* and *C. difficile* have been isolated from blood and peritoneal cultures of infants with necrotising enterocolitis and with sudden death syndrome.⁸ *Clostridium* spp. have also been isolated from children with bacteraemia of gastrointestinal origin¹ and with sickle cell disease.⁹ *C. difficile* is the causative agent of antibiotic-associated and spontaneous diarrhoea and pseudomembranous colitis in adults.^{10,11} Infants are not susceptible to intestinal infection with *C. difficile*, but there were three isolates from bacteraemia or peritonitis in this study and its isolation has been reported in children from infections of the peritoneal cavity, blood and lungs, and from wounds and central nervous system infections.¹

Clostridia can act synergically with other aerobic and anaerobic bacteria.¹² As a result, polymicrobial infections that involve clostridia may be more destructive and more difficult to eradicate. The synergy between the different bacterial strains may be due to inhibition of phagocytosis and intracellular killing,¹³ production of essential growth factors,¹⁴ or lowering of redox potentials in host tissues.¹⁵

C. botulinum is usually associated with food poisoning;^{1,4} botulism is an intoxication caused by ingestion of contaminated food containing its highly potent neurotoxin. However, wound infections caused by proteolytic strains of types A and B have been reported with increasing frequency and can also produce botulism. This was not observed in the present study.

The management of clostridial infections comprises a combination of antimicrobial therapy and surgical intervention. Drainage of pus and debridement of necrotic tissues are integral to therapy. Most infections from which clostridia are isolated require antimicrobial therapy. Clostridia are generally susceptible to most antibiotics used for the treatment of anaerobic infections,¹⁶ including β -lactam antibiotics, macrolides, carbapenems, imidazoles and chloramphenicol. Susceptibility to antimicrobial agents was not recorded in the present study, but general guidelines can be based upon previously reported susceptibility data.

Increased resistance of clostridia to several anti-

microbial agents has been reported by others.¹⁷⁻²⁰ A survey of isolates¹⁷ from the Mayo Clinic described multi-resistant *Clostridium* spp. Isolates of *C. clostridiiforme*, *C. innocuum* and *C. ramosum* were resistant to achievable serum concentration "break-points" of chloramphenicol (10-33%), clindamycin (23-30%), penicillin (16-26%), metronidazole (11-12%) and cefoxitin (22-48%). Isolates of *C. butyricum* that produced β -lactamase and were resistant to penicillins have been described.^{17,18} These investigators also noted that a minority of *C. perfringens* strains were resistant to clindamycin (14%) and penicillin (5%). Marrie *et al.*²⁰ also reported increased resistance to penicillin in *C. perfringens* isolates in Canada; 50% of isolates were inhibited by 0.25 mg/L but the MIC₉₀ was 8 mg/L. The susceptibility of *Clostridium* spp. needs to be monitored constantly to ensure correct empiric therapy.

I gratefully acknowledge the technical support of L. Calhoun, P. Yocum, J. Perry and E. H. Frazier, and the editorial assistance of Sarah Blaisdell.

References

1. Brook I. Pediatric anaerobic infections: diagnosis and management. A textbook. Philadelphia, PA, Mosby Publisher, Inc. 1989.
2. Sutter VL, Citron DM, Edelstein MAC, Finegold SM. Wadsworth bacteriology manual, 4th edn. Belmont, CA, Star Publishing Co. 1985.
3. Holdeman LV, Cato EP, Moore WEC. Anaerobe laboratory manual, 4th edn. Blacksburg, VA, Virginia Polytechnic Institute and State University. 1977.
4. Finegold SM. Anaerobic bacteria in human disease. New York, Academic Press. 1977.
5. Rosebury T. Microorganisms indigenous to man. New York, McGraw-Hill Book Co. 1966.
6. Brook I. Recovery of anaerobic bacteria from clinical specimens in 12 years at two military hospitals. *J Clin Microbiol* 1988; **26**: 1181-1188.
7. Howard MF, Flynn DM, Bradley JM, Noone P, Szawatkowski M. Outbreak of necrotising enterocolitis caused by *Clostridium butyricum*. *Lancet* 1977; **2**: 1099-1102.
8. Cashore WJ, Peter G, Lauermann M, Stonestreet BS, Oh W. *Clostridia* colonization and clostridial toxin in neonatal necrotizing enterocolitis. *J Pediatr* 1981; **98**: 308-311.
9. Brook I, Gluck RS. *Clostridium paraprutificum* sepsis in sickle cell anaemia. *South Med J* 1980; **73**: 1644-1645.
10. Viscidi RP, Bartlett JG. Antibiotic-associated pseudomembranous colitis in children. *Pediatrics* 1981; **67**: 381-386.
11. Brook I. Isolation of toxin producing *Clostridium difficile* from two children with oxacillin- and dicloxacillin-associated diarrhea. *Pediatrics* 1980; **65**: 1154-1156.
12. Brook I, Hunter V, Walker RI. Synergistic effect of *Bacteroides*, *Clostridium*, *Fusobacterium*, anaerobic cocci, and aerobic bacteria on mortality and induction of subcutaneous abscesses in mice. *J Infect Dis* 1984; **149**: 924-928.
13. Ingham HR, Tharagannet D, Sisson PR, Selkon JB, Codd AA. Inhibition of phagocytosis *in vitro* by obligate anaerobes. *Lancet* 1977; **2**: 1252-1254.
14. Lev M, Keudell KC, Milford AF. Succinate as a growth factor for *Bacteroides melaninogenicus*. *J Bacteriol* 1971; **108**: 175-178.
15. Mergenhagen SE, Thonard JC, Scherp HW. Studies on synergistic infections. I. Experimental infections with anaerobic streptococci. *J Infect Dis* 1958; **103**: 33-44.
16. Sutter VL, Finegold SM. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. *Antimicrob Agents Chemother* 1976; **10**: 736-752.
17. Edson RS, Rosenblatt JE, Lee DT, McVey EA. Recent experience with antimicrobial susceptibility of anaerobic bacteria: increasing resistance to penicillin. *Mayo Clin Proc* 1982; **57**: 737-741.
18. Carlson JR, Sherrill JM, Rosenblatt JE, McCarthy LR. Penicillinase activity in three strains of *Clostridium butyricum*. *Curr Microbiol* 1981; **5**: 251-254.
19. Brazier JS, Levett PN, Stannard AJ, Phillips KD, Willis AT. Antibiotic susceptibility of clinical isolates of clostridia. *J Antimicrob Chemother* 1985; **15**: 181-185.
20. Marrie TJ, Haldane EV, Swantee CA, Kerr EA. Susceptibility of anaerobic bacteria to nine antimicrobial agents and demonstration of decreased susceptibility of *Clostridium perfringens* to penicillin. *Antimicrob Agents Chemother* 1981; **19**: 51-55.