

Isolation of non-sporing anaerobic rods from infections in children

I. BROOK*

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

From 1974 to 1994, 2033 microbiological specimens from children were submitted for cultures for anaerobic bacteria. Fifty-seven isolates of *Bifidobacterium* spp. were obtained from 55 (3%) children, 67 isolates of *Eubacterium* spp. from 65 (3%) children and 41 isolates of *Lactobacillus* spp. from 40 (2%) children. Most *Bifidobacterium* isolates were from chronic otitis media, abscesses, peritonitis, aspiration pneumonia and paronychia. Most *Eubacterium* isolates were from abscesses, peritonitis, decubitus ulcers and bites. *Lactobacillus* spp. were mainly isolated from abscesses, aspiration pneumonia, bacteraemia and conjunctivitis. Most (> 90%) infections from which these species were isolated were polymicrobial and yielded a mixture of aerobic and anaerobic bacteria. The organisms most commonly isolated with the non-sporing anaerobic gram-positive rods were *Peptostreptococcus* spp., *Bacteroides* spp., pigmented *Prevotella* and *Porphyromonas* spp., *Fusobacterium* spp., *Staphylococcus aureus* and *Escherichia coli*. Most *Bacteroides* spp. and *E. coli* were isolated from intra-abdominal infection and skin and soft tissue infection around the rectal area, whereas most *Prevotella*, *Porphyromonas* and *Fusobacterium* isolates were from oropharyngeal, pulmonary and head and neck sites. The predisposing conditions associated with the isolation of non-sporing anaerobic gram-positive rods were previous surgery, malignancy, steroid therapy and immunodeficiency. Antimicrobial therapy was given to 149 (83%) of the 160 patients, in conjunction with surgical drainage or correction of pathology in 89 (56%).

Introduction

There has been increased interest in recent years about the role of anaerobic bacteria in paediatric infections [1]. The anaerobic species of most concern have been members of the genera *Bacteroides*, *Clostridium*, *Fusobacterium* and *Peptostreptococcus* [1]. Non-sporing, anaerobic, gram-positive rods such as *Bifidobacterium*, *Eubacterium* and *Lactobacillus* species are usually considered to be of relatively low virulence. These organisms are taxonomically unrelated but are all non-motile and catalase-negative. These organisms have been isolated from adults with pulmonary infection (*B. dentium*) [2], from intra-uterine devices (*E. nodatum*) [3], and from peritonitis and the bloodstream (*Lactobacillus* spp.) [4, 5]. However, their pathogenic role in these and other infections is uncertain. The organisms have been isolated in pure culture in only a few instances and are usually isolated in mixed culture from clinical specimens [1, 6]. The

infections where they have been found most often are chronic otitis media and sinusitis, aspiration pneumonia, and intra-abdominal, obstetric and gynaecological and skin and soft-tissue infections [1, 6, 7].

This retrospective review summarises my experience of the isolation of *Bifidobacterium*, *Eubacterium* and strictly anaerobic *Lactobacillus* spp. from infections in children over a 20-year period. Some of the data have been published previously in articles describing the role of anaerobic bacteria in various paediatric infections [1], but cases not previously presented are also included, and the clinical associations of *Bifidobacterium*, *Eubacterium* and strictly anaerobic *Lactobacillus* spp. in infections in children are emphasised.

Patients and methods

Patients

The specimens included in this review were submitted for anaerobic investigation between June 1974 and June 1994 in the following hospitals: University of California Medical Center, County Medical Center and Serra

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

Memorial Hospital in Los Angeles, and Fairview State Hospital, Costa Mesa, CA; Children's Hospital National Medical Center and South-East Community Medical Center in Washington DC; and the Naval Hospital in Bethesda, MD, USA. The clinical microbiology laboratory records were reviewed to identify patients from whom *Bifidobacterium*, *Eubacterium* and anaerobic *Lactobacillus* spp. were isolated and reported as potentially significant pathogens. Where available, the case records of the patients were reviewed to ascertain the presence and site of infection, associated micro-organisms, underlying diseases and possible predisposing or associated conditions.

Microbiological examination

Only specimens that were collected properly without contamination by the normal skin or mucosal flora and submitted in transport media appropriate for anaerobic bacteria were accepted by the microbiology laboratories. These were generally specimens obtained during surgery or by aseptic needle or biopsy aspiration of abscesses or fluid from body cavities. Lung aspirates were obtained by transtracheal aspiration or through a tracheostomy or endotracheal tube or by biopsy. When possible, pus and fluids were collected and transported in syringes. Tissues were transported in oxygen-free gassed-out tubes. Swab specimens were submitted in the Port-A-Cul transport swab system (BBL, Cockeysville, MD, USA). However, precise records of all of the transport media used were not available. Blood for culture was collected aseptically from patients suspected of having bacteraemia and was inoculated (10%) into the anaerobic bottle of one of two commercially produced blood culture broth media; both were under vacuum and with CO₂ 5% in the atmosphere.

The specimens were inoculated on to pre-reduced vitamin K₁-enriched *Brucella* Blood Agar (BBL), blood agar with kanamycin and vancomycin, blood agar containing colistin sulphate and nalidixic acid, and an enriched thioglycolate broth containing haemin and vitamin K₁ [8, 9]. The cultures were incubated in GasPak jars (BBL) and examined after 48 and 96 h [9]. Plates that showed any growth were held until the micro-organisms had been identified. All cultures that showed no growth were incubated for at least 5 days before being discarded. Anaerobic isolates were identified by the API Anaerobic System (Analytab Products, Plainview, NY, USA) or by the Minitab system (BBL). When complete identification was not possible by these methods, other carbohydrate tests (Scott Laboratories, Fiskeville, RI, USA) and gas-liquid chromatography (GLC) [8, 9] were performed as needed to identify the organisms. The criteria for identification were according to guidelines in published schemes [8–10].

In gram-stained films, *Bifidobacterium* spp. are

generally branched or bifurcate, *Eubacterium* spp. are pleomorphic and *Lactobacillus* spp. are straight or curved. Species vary from strictly anaerobic to aerotolerant [8, 9]. *Bifidobacterium* and *Lactobacillus* spp. are nitrate-positive and indole-negative. *Lactobacillus* spp. produce lactic acid as the sole end product whereas *Bifidobacterium* spp. produce acetic and lactic acids (with more of the former than the latter). The genus *Eubacterium* comprises gram-positive, non-sporing bacilli whose GLC pattern is not characteristic of other genera.

Results

During the study period, 2033 specimens were examined for anaerobic bacteria; 57 isolates of *Bifidobacterium* spp., 67 of *Eubacterium* spp. and 41 of strictly anaerobic *Lactobacillus* spp. were obtained from various sites that accounted for 1780 specimens. These species were not isolated from other sites, such as bile, joint, bone, sinuses, central nervous system and urinary tract, that accounted for 253 specimens.

Bifidobacterium spp.

The 57 *Bifidobacterium* isolates were from 55 patients aged 2 weeks–16 years (mean 5 years and 8 months); 37 were males (Table 1). The isolates comprised 19 (33%) *B. adolescentis*, five (9%) *B. dentium* and 33 (58%) other *Bifidobacterium* spp. Infections were polymicrobial in 53 (96%) patients, but in two (4%) a *Bifidobacterium* spp. was isolated in pure culture; these were an isolate of *B. adolescentis* from chronic otitis media and a *Bifidobacterium* sp. from cervical adenitis.

Most *Bifidobacterium* isolates were from chronic otitis media (23; 40%), abscesses or peritonitis (7; 12% each), cholesteatoma, aspiration pneumonia and paronychia (3; 5% each). In the 53 specimens that yielded mixed growth including a *Bifidobacterium* sp., there were 148 other isolates; 89 (60%) of these were strict anaerobes and 59 (40%) were facultative or aerobic species. The number of isolates in mixed cultures varied from two to five (average 2.8 isolates/specimen; 1.7 anaerobes and 1.1 facultative or aerobic species).

The anaerobic organisms isolated most commonly with *Bifidobacterium* spp. were *Peptostreptococcus* spp. (28 isolates), *Fusobacterium* spp. (17), pigmented *Prevotella* and *Porphyromonas* spp. (16) and *Bacteroides* spp. (6). The most common aerobic and facultative organisms isolated with *Bifidobacterium* spp. were α -haemolytic streptococci (15 isolates), *Staphylococcus aureus* (9), *Escherichia coli* (7), *Pseudomonas aeruginosa* (5) and *Streptococcus pyogenes* (5). Most *Bacteroides* and *E. coli* isolates found in mixed culture with *Bifidobacterium* spp. were from peritonitis, whereas most *Prevotella*, *Porphyromonas*

Table 1. Significant isolates of *Bifidobacterium*, *Eubacterium* and *Lactobacillus* from clinical specimens in children

Type of infection	Total specimens examined	Number of isolates of												
		<i>Bifidobacterium</i>				<i>Eubacterium</i>								
		<i>B. dentium</i>	<i>B. adolescentis</i>	<i>Bifidobacterium</i> spp.	Total	<i>E. tenue</i>	<i>E. multiforme</i>	<i>E. moniliforme</i>	<i>E. limosum</i>	<i>E. lentum</i>	<i>Eubacterium</i> spp.	Total	<i>L. minutus</i>	<i>L. jensenii</i>
Abscess	428		1	6	7			1	1	2	24	28		2
Bacteraemia	342													
Cervical lymphadenitis	53		1	1	2									
Ears: chronic otitis media	142	5	7	11	23									
chronic mastoiditis	24								2			2		
cholesteatoma	24		1	2	3									
Pulmonary														
aspiration pneumonia	74		1	2	3	1						1		
lung abscess	10		1	1	2									
pneumonia in cystic fibrosis	6													
Tracheostomy site	25			1	1						2	2		
Paronychia	33		2	1	3	1					1	2		
Wounds	75										3	3		
Burns	180			2	2				2			2		
Bites	39		1	1	2				1	1	1	3		
Gastrostomy site	22					1						1		
Decubitus ulcers	58					2	1				1	4		
Peritonitis	116		4	3	7					7	10	17		
Conjunctivitis	129			2	2	1				1		2		
Total	1780	5	19	33	57	6	1	1	6	11	42	67		2

and *Fusobacterium* spp. were from ear, head and neck and pulmonary sites. Anaerobic cocci were isolated from all sites.

Eleven (20%) of the patients had predisposing or underlying medical or surgical conditions. These were previous surgery (5), malignancy (2), steroid therapy (2) and tympanostomy tubes (2). Antimicrobial therapy was administered to all patients, in conjunction with surgical drainage with or without correction of pathology in 21 (38%). The infections resolved in 49 (89%) of the patients. Six (29%) of the 21 cases with chronic otitis media did not resolve. The systemic antimicrobial agents given were clindamycin (in 16 instances), a cephalosporin (14), oxacillin (8), co-amoxyclov (8), gentamicin (7), penicillin (4) and chloramphenicol (3). Local therapy with erythromycin ointment was used to treat the two cases of conjunctivitis.

Eubacterium spp.

The 67 isolates of *Eubacterium* spp. were from 65 patients aged 7 days–16 years (mean 6 years and 2 months); 39 patients were male (Table 1). The isolates comprised 11 (19%) *E. lentum*, six (11%) each of *E. limosum* and *E. tenue*, and one (2%) each of *E. moniliforme* and *E. multiforme* and 42 (74%) *Eubacterium* spp. Infections were polymicrobial in 61 (94%) patients, but in four (6%) a *Eubacterium* sp. was the only isolate. The four isolates found in pure culture were one each of *E. lentum* and *Eubacterium* sp. in subcutaneous abscesses, and one isolate of a *Eubacterium* sp. in each of a wound and a burn site.

Most *Eubacterium* isolates were from abscesses (28; 41%), peritonitis (17; 25%), decubitus ulcers (4; 6%) and bites (3; 4%). The 26 abscesses from which eubacteria were isolated were peritonsillar (8), retropharyngeal (4), perirectal (4), dental (3), abdominal (3), subcutaneous (2) and scalp (2). In the 61 specimens that yielded a mixed growth including a *Eubacterium* sp., there were 152 other isolates; 94 (62%) of these were strict anaerobes and 58 (38%) were facultative or aerobic bacteria. The number of isolates in mixed infection cultures varied from two to six (average 2.5 isolates/specimen; 1.5 anaerobes and 1.0 facultative or aerobic). The anaerobic organisms isolated most commonly with eubacteria were *Peptostreptococcus* spp. (40 isolates), *Bacteroides* spp. (16), *Prevotella* and *Porphyromonas* spp. (15) and *Fusobacterium* spp. (6). The most common aerobic and facultative organisms isolated with *Eubacterium* spp. were *S. aureus* (16 isolates), *Esch. coli* (13), α -haemolytic streptococci (12) and *Str. pyogenes* (5). Most *Bacteroides* spp. and *E. coli* found in mixed culture with *Eubacterium* spp. were from peritonitis and abscesses and wounds around the rectal area. Most *Prevotella*, *Porphyromonas* and *Fusobacterium*

spp. were isolated from wounds and abscesses around or in the oropharynx.

Twelve (18%) of the children had predisposing or underlying medical or surgical conditions. These were previous surgery (4), malignancy (3), steroid therapy (2), diabetes (2) and sickle cell disease (1). Systemic antimicrobial therapy was given to 59 (91%) patients, in conjunction with surgical drainage with or without correction of pathology in 47 (72%) instances. All the infections resolved. The antimicrobial agents given were clindamycin (in 18), a cephalosporin (16), gentamicin (12), oxacillin (9), co-amoxyclov (8), penicillin (6), chloramphenicol (4), erythromycin (3) and ticarcillin-clavulanate (2). Local therapy with an antibiotic or anti-infective ointment was given to two patients with wounds, two with burns and two with conjunctivitis.

Lactobacillus spp.

The 41 isolates of *Lactobacillus* spp. were from 40 children aged 8 months–16 years (mean 7 years and 5 months). The isolates were five (12%) *L. acidophilus*, three (7%) *L. fermentum*, two (5%) *L. jensenii*, one (2%) *L. catenaforme* and 30 (75%) *Lactobacillus* spp. Infections were polymicrobial in 36 (90%) patients. The four *Lactobacillus* isolates from blood cultures were in pure culture and were from four patients with intravenous catheter-related bacteraemia.

Most *Lactobacillus* isolates were from abscesses (14; 34%), aspiration pneumonia (8; 20%), ear infection, bacteraemia and conjunctivitis (4; 10% each). The 14 abscesses from which *Lactobacillus* spp. were isolated were dental (4), subcutaneous (3), vulvovaginal (2), abdominal (2), subdiaphragmatic (1), lung (1) and tonsillar (1). In the 36 specimens that yielded mixed growth including a *Lactobacillus* sp., there were 76 other isolates; 49 (64%) of these were strict anaerobes and 27 (36%) were facultative or aerobic bacteria. The number of isolates in mixed cultures varied from two to five (average 2.1 isolates/specimen, 1.4 anaerobes and 0.7 facultative or aerobic). The anaerobic organisms isolated most often with *Lactobacillus* spp. were *Peptostreptococcus* spp. (20 isolates), pigmented *Prevotella* and *Porphyromonas* spp. (15) and *Fusobacterium* spp. (8). The most common aerobic and facultative organisms isolated with *Lactobacillus* spp. were *S. aureus* (7 isolates), α -haemolytic streptococci (6), *Haemophilus influenzae* (3) and *E. coli* (2). All *Prevotella*, *Porphyromonas* and *Fusobacterium* spp. were from abscesses and wounds around or in the oropharynx. Most *S. aureus* isolates were from subcutaneous abscesses and wounds, and the *H. influenzae* isolates were from conjunctivitis and otitis.

Eight of the patients had one or two predisposing or underlying medical or surgical conditions. These were intravenous catheter (4), immunodeficiency (3), ma-

absorption (2) and malignancy (1). Systemic antimicrobial therapy was given to 35 (87%) patients, in conjunction with drainage with or without correction of pathology in 21 (52%) instances. All the infections resolved. The antimicrobial agents used were clindamycin (12), gentamicin (10), amoxicillin (9), co-amoxiclav (7), a cephalosporin (7), ticarcillin-clavulanate (4) and methicillin (3). In addition to systemic antimicrobial therapy, two of the four catheters associated with bacteraemia were removed. The two other patients recovered without the removal of the catheters. Local therapy with ophthalmic ointment containing antimicrobial agents resulted in recovery in the four cases of conjunctivitis.

Discussion

This review demonstrates the prevalence of *Bifidobacterium*, *Eubacterium* and *Lactobacillus* spp. in various infections in children. The 57 *Bifidobacterium* isolates were obtained from 55 (3%) of the 2033 specimens submitted for anaerobic cultures, the 67 isolates of *Eubacterium* spp. were from 65 (3%) patients and the 41 *Lactobacillus* isolates were from 40 (2%) children. These micro-organisms were found most commonly in infections associated with predisposing or underlying conditions such as previous surgery, malignancy, immunodeficiency and the presence of a foreign body.

All three genera of non-sporing gram-positive anaerobic rods were found in abscesses. *Bifidobacterium* spp. were found mostly in head and neck and pulmonary infections, and in a smaller number of cases of peritonitis. In contrast, *Eubacterium* spp. were not isolated from chronic otitis media but were more frequent in peritonitis and wounds. *Lactobacillus* spp. were most commonly found in aspiration pneumonia and bacteraemia associated with intravenous catheters.

Species of the genus *Bifidobacterium* are part of the commensal flora of the mouth, gastrointestinal tract and female genital tract and constitute a high proportion of the normal intestinal flora in man, especially in breast-fed infants [11]. Although some infections caused by these organisms have been reported [12–15], little is known about their pathogenic potential. *Eubacterium* spp. are part of the flora of the mouth and the bowel. They have been recognised as pathogens in chronic periodontal disease [16], and in infections associated with intra-uterine devices [3], and have been isolated from patients with bacteraemia associated with malignancy [17] and from female genital tract infection [18]. *Lactobacillus* spp. are ubiquitous inhabitants of the human oral cavity, the vagina and the gastrointestinal tract [19]. They have been implicated in various serious deep-seated infections, amnionitis [19] and bacteraemia [20].

The isolation of non-sporing anaerobic gram-positive rods from sites of sometimes serious infections in children suggests that they should not be automatically dismissed as contaminants. It appears that they may have clinical significance in high-risk patients with serious underlying illnesses. As they can cause significant infections, especially in high-risk patients, efforts should be made to secure specimens free of contamination by the normal flora of the mucous membranes and the skin, and the clinical significance of each isolate must be carefully evaluated. Eradication of these micro-organisms from deep-seated sites of infection may be difficult. All non-sporing anaerobic gram-positive rods are susceptible to penicillin G, carbenicillin and chloramphenicol [21]. Clindamycin is effective against 94% of isolates, erythromycin against 88% and tetracycline against 60% [22]. Metronidazole is effective against only 50% of isolates [21–23]. The efficacy of cephalosporins varies between 89% and 95%.

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