CLINICAL MICROBIOLOGY

Aerobic and anaerobic microbiology in intra-abdominal infections associated with diverticulitis

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The aerobic and anaerobic microbiology of intra-abdominal infections associated with diverticulitis was studied in 110 specimens from the peritoneal cavity after intestinal perforation and in 22 specimens from abdominal abscesses. Anaerobic bacteria only were isolated from 17 (15%) of the peritoneal specimens, aerobic bacteria only from 12 (11%) and mixed aerobic and anaerobic flora from 81 (74%). A total of 339 bacterial isolates was detected in peritoneal cultures (3.1 per specimen), comprising 155 aerobes (1.4 per specimen) and 184 anaerobes (1.7 per specimen). Anaerobic bacteria only were isolated in 4 (18%) abscesses, aerobes alone in one (5%) and mixed aerobic and anaerobic flora in 17 (77%). A total of 72 bacterial isolates (3.3 per specimen) was detected in abdominal abscesses – 35 aerobes (1.6 per specimen) and 37 aerobes (1.7 per specimen). The predominant aerobic and facultative bacteria in abdominal infections were *Escherichia coli* and *Streptococcus* spp. The most frequently isolated anaerobes were *Bacteroides* spp. (*B. fragilis* group), *Peptostreptococcus*, *Clostridium* and *Fusobacterium* spp.

Introduction

The microbiology of intra-abdominal infections after perforation of the viscus has been established by numerous studies [1–7]. Polymicrobial aerobic and anaerobic organisms were found to predominate in the majority of patients, with an average number of isolates of approximately five per infection site [1–7]. Generally, the specific micro-organisms involved in these infections are those of the normal flora of the gastrointestinal tract, in which anaerobes reach a concentration of 10^{9-11} organisms/ml and outnumber aerobes 1000:1 [8].

Previous studies of intra-abdominal infections described the microbiological findings in peritonitis or abscesses that occurred after perforation of the appendix [1], or combined all other causes of perforation of the viscus [2–7]. Although some studies included infections associated with diverticulitis [6, 7],

none defined the specific findings following this condition.

This retrospective study analysed the aerobic and anaerobic microbiology of intra-abdominal infections (peritonitis and intra-abdominal abscesses) that were associated with diverticulitis in a tertiary care military hospital during a 15-year period.

Patients and methods

Specimens included in the analysis were obtained from patients who developed their infection as a complication of diverticulitis between June 1978 and June 1993; 132 clinical specimens of peritoneal fluid, and 27 specimens of pus from abdominal abscesses were submitted to the clinical microbiological laboratory at the Navy Medical Center in Bethesda, Maryland. Bacterial growth was present in 113 (89%) peritoneal fluid specimens and 25 (93%) abscesses. However, the final analyses was based on the 110 patients with peritonitis and 22 with abdominal abscesses whose clinical data were available for analysis. Patients' ages ranged from 42 to 84 years (average 57.2 years), and 69 patients with peritonitis and 18 with abscesses were male.

Antimicrobial agents were given to 79 patients with

Received 22 Feb. 2000; accepted 10 March 2000.

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peritonitis and 18 with abscesses before collection of samples. These antimicrobial agents included a penicillin (28 patients), a cephalosporin (41), an aminoglycoside (30), clindamycin (21), metronidazole (8), imipenem (5) and erythromycin (2).

Specimens submitted to the microbiology laboratory were obtained through an open surgical procedure, either by aspiration of the pus into a syringe that was sealed with a rubber stopper after evacuation of the air or by a swab that was transported in an anaerobic transport tube (Port-A-Cul; Becton Dickinson, Sparks, MD, USA). The time between collection of material and inoculation of specimens ranged from 30 min to 2 h.

Specimens were inoculated on to sheep blood 5%, chocolate and MacConkey agar plates for aerobes and facultative organisms. The plates were incubated aerobically at 37°C (MacConkey) or in air with CO₂ 5% (sheep blood 5% and chocolate) and examined at 24 and 48 h. For anaerobes, the material was plated on to pre-reduced brucella blood agar enriched with vitamin K₁, an anaerobic blood agar plate containing kanamycin and vancomycin, an anaerobic blood agar plate containing phenylethyl alcohol and an enriched thioglycolate broth containing haemin and vitamin K₁ [9]. The anaerobic plates and thioglycolate broth were incubated in GasPak jars (Baltimore Biological Laboratories, Baltimore, MD, USA) and examined at 48 and 96 h. Anaerobes were identified by techniques described previously [9]. Aerobic bacteria were identified by conventional methods [10].

Blood was drawn, most often from an antecubital vein,

after preparation of the area with povidone/iodine, and it was inoculated at the bedside into two bottles, one supportive of growth of aerobic bacteria and the other supportive of anaerobic bacterial growth.

Results

Peritoneal cavity

Anaerobic bacteria alone were present in 17 (15%) of 110 specimens, aerobes alone in 12 (11%) and mixed aerobic and anaerobic flora in 81 (74%). A total of 339 bacterial isolates was obtained from the specimens (3.1 per specimen) (Tables 1 and 2), comprising 155 aerobic or facultative organisms (1.4 per specimen) and 184 anaerobes (1.7 per specimen).

The most frequently isolated aerobic or facultative organisms were *Escherichia coli*, γ -haemolytic streptococci, α -haemolytic streptococci, group D streptococci, *Klebsiella* spp. and *Pseudomonas aeruginosa*. The predominant anaerobes were *Bacteroides* spp. (*B. fragilis* group), *Peptostreptococcus*, *Clostridium*, *Fusobacterium* and *Prevotella* spp., and *Porphyromonas asaccharolytica*. Polymicrobial infection occurred in 103 (94%) instances. No consistent patterns of bacterial combinations were noted except between the *Bacteroides* spp. and *E. coli* in 37 instances.

Abscesses

Anaerobic bacteria alone were present in 4 (18%) of 22 specimens, aerobes alone in 1 (5%) and mixed aerobic and anaerobic flora in 17 (77%). In all, there were 72 bacterial isolates (3.3 isolates per specimen) – 37

Table 1. Aerobic and facultative organisms isolated from patients with intra-abdominal infections associated with diverticulitis

Organism	Number of isolates from	
	Peritoneal fluid (n = 110)	Intra-abdominal abscess (n = 22)
Gram-positive cocci		
α -Haemolytic streptococci	12	6
γ -Haemolytic streptococci	14	2
Group D streptococci	11	3
Group A, β -haemolytic streptococci	3	...
Group C, β -haemolytic streptococci	2	...
Group F, β -haemolytic streptococcus	1	...
<i>Staphylococcus epidermidis</i>	2	...
<i>S. aureus</i>	1	1
Gram-positive bacilli		
<i>Corynebacterium</i> spp.	2	...
Gram-negative bacilli		
<i>Haemophilus</i> spp.	1	...
<i>Eikenella corrodens</i>	2	...
<i>Pseudomonas aeruginosa</i>	7	1
<i>Escherichia coli</i>	72	15
<i>Klebsiella pneumoniae</i>	8	3
<i>K. oxytoca</i>	4	...
<i>Enterobacter</i> spp.	3	...
<i>Serratia marcescens</i>	2	1
<i>Proteus</i> spp.	3	1
Enterobacteriaceae (other)	5	2
Total number of aerobic and facultative isolates	155	35

Table 2. Anaerobic organisms isolated from patients with intra-abdominal infections associated with diverticulitis

Organism	Number of isolates	
	Peritoneal fluid (n = 110)	Intra-abdominal abscess (n = 22)
Gram-positive cocci		
<i>Peptostreptococcus</i> spp.	8	2
<i>Pstr. magnus</i>	3	2
<i>Pstr. asaccharolyticus</i>	3	1
<i>Pstr. prevotii</i>	2	...
<i>Pstr. micros</i>	3	...
<i>Pstr. anaerobius</i>	2	1
<i>Pstr. morbilorum</i>	2	...
<i>Streptococcus intermedius</i>	1	...
Microaerophilic streptococci	1	...
Gram-negative cocci		
<i>Veillonella parvula</i>	2	1
<i>V. alcalescence</i>	1	...
Gram-positive bacilli		
<i>Bifidobacterium</i> spp.	1	...
<i>Eubacterium</i> spp.	4	...
<i>Eu. lentum</i>	7	1
<i>Lactobacillus</i> spp.	2	...
<i>Propionibacterium acnes</i>	6	...
<i>Clostridium</i> spp.	12	3
<i>C. perfringens</i>	10	2
<i>C. ramosum</i>	4	...
<i>C. butyricum</i>	1	...
<i>C. clostridiiforme</i>	2	...
<i>C. septicum</i>	2	...
Gram-negative bacilli		
<i>Fusobacterium</i> spp.	7	1
<i>F. varium</i>	1	...
<i>F. necrophorum</i>	2	...
<i>F. nucleatum</i>	3	...
<i>Bacteroides</i> spp.	10	1
<i>B. fragilis</i>	44	14
<i>B. ovatus</i>	7	2
<i>B. vulgatus</i>	7	...
<i>B. distasonis</i>	6	1
<i>B. thetaiotaomicron</i>	11	2
<i>B. uniformis</i>	1	...
<i>Porphyromonas asaccharolytica</i>	2	1
<i>Prevotella intermedia</i>	1	...
<i>Pr. melaninogenica</i>	2	2
<i>Pr. bivia</i>	1	...
Total number of anaerobic isolates	184	37

anaerobes (1.7 per specimen) and 35 aerobic or facultative anaerobes (1.6 per specimen) (Tables 1 and 2). The predominant aerobic and facultative organisms were *E. coli* and *Streptococcus* spp. The most frequently isolated anaerobes were *Bacteroides* spp., *Peptostreptococcus* and *Clostridium* spp. Polymicrobial flora were recovered in 19 (86%) patients. No consistent patterns of bacterial combinations were noted except between the *Bacteroides* spp. and *E. coli* in 11 instances.

Sixteen organisms identical to that isolated from the peritoneal cavity and seven similar to those found in the abscesses were also isolated from blood cultures in 13 (17%) of the 76 cases of peritonitis and 5 (33%) of the 15 cases of abscesses in which blood cultures were performed. These organisms were *E. coli* (7), *Bacteroides* spp. (5), *Peptostreptococcus* spp. (4), *Klebsiella pneumoniae* (3), *Fusobacterium* spp. (2) and *Clostridium* spp. (2).

Discussion

This study demonstrates the presence of mixed aerobic and anaerobic flora in secondary peritonitis and intra-abdominal abscesses associated with diverticulitis. These data conform with those of previous studies of intra-abdominal infection due to ruptured appendix [1] and other causes of rupture of viscus [2–7], in which *B. fragilis*, *E. coli* and *Peptostreptococcus* spp. were the predominant pathogens. The findings of similar micro-organisms in intra-abdominal infections associated with diverticulitis as in infections due to other causes of rupture of the viscus is not surprising, as the origin of all these infections is the gastrointestinal flora.

The importance of the aerobic and anaerobic components of these infections has been demonstrated in experimental studies of animals [11]. *B. fragilis*, the most prevalent among the anaerobes isolated, has

several virulence factors. These include resistance to β -lactam antibiotics through production of the enzyme β -lactamase [12], elaboration of other enzymes and byproducts and possession of a capsule that inhibits phagocytosis [13].

The relationship between the aerobic and anaerobic bacteria in these infections has been shown to be synergic [11]. Several hypotheses have been proposed to explain such microbial synergy. It may be a result of mutual protection from phagocytosis and intracellular killing [13], production of essential growth factors [14] or lowering of oxidation-reduction potentials in host tissues [15].

This study underscores the importance of obtaining specimens for the investigation of both aerobic and anaerobic bacteria from abdominal infections associated with diverticulitis. Management of mixed aerobic and anaerobic infections requires, in addition to surgical correction and drainage of pus, the administration of antimicrobial agents effective against both aerobic and anaerobic bacterial components of the infection [16–18]. When such therapy is not given, the infection may persist and a complication such as an abscess may occur.

The environment of an abscess is detrimental to many antimicrobial agents. The abscess capsule, the low pH and the presence of binding proteins or inactivating enzymes such as β -lactamase may impair the activity of many agents [19]. Because of these limitations, drainage is still the therapy of choice when abscesses have already developed.

We acknowledge the efforts of the staffs of the clinical microbiology laboratories and the clinical wards at the Navy Hospital in Bethesda and the secretarial assistance of Sarah Blaisdell.

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