Anaerobes in Human Biliary Tracts

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During a 2-year period, 1,892 patients underwent biliary tract surgery at the Mayo Clinic. Both aerobic and anaerobic cultures of bile were performed in 371 of these patients. Sixty-nine percent of the cultures were positive, and 41% (117) of these grew anaerobes, although they were present in pure culture only twice. Mixed cultures most commonly contained four different organisms (three aerobes and one anaerobe). *Bacteroides fragilis* was the single most commonly isolated anaerobe and ranked fourth in terms of overall isolates behind *Escherichia coli*, group D streptococci, and *Klebsiella*. *B. fragilis* accounted for 7.0% of the total aerobic and anaerobic isolates and was present in 21% of all positive cultures. *Pseudomonas aeruginosa* and *Clostridium perfringens* ranked fifth and sixth, providing 6.5 and 5.9% of all isolates, respectively. This study demonstrates the frequent presence of anaerobes in patients with bactibilia and suggests that they be considered in the formulation of antimicrobial therapy for infections involving human biliary tracts.

Colonization of human biliary tracts with aerobic bacteria has been reported in 25 to 50% of patients cultured (4, 8, 10, 11, 14). (In this report, the term "aerobic" is used to include both those organisms that are obligately aerobic and those that are facultatively anaerobic.) The organisms most often isolated include *Escherichia coli*, *Klebsiella*, group D streptococcus, *Enterobacter*, and *Staphylococcus epidermidis*. Aerobic bacteria are isolated from biliary tracts with increased frequency in elderly patients (8, 12, 17, 19) and in the following types of biliary tract diseases: acute emphysematous cholecystitis (8, 11, 16, 17), obstruction of the common bile duct with choledocholithiasis (5, 8, 12), and after multiple, previous biliary tract operations (1, 15, 20). Aerobic bactibilia has been reported to be polymicrobial in 17 to 50% of cultures (8, 9, 12, 17, 19). The organisms most commonly found in these studies are similar to those listed above. Studies of experimental biliary tract infections in animals have implicated *E. coli* as the primary pathogen (13).

The role of anaerobic bacteria in colonization and infection of biliary tracts has been much less studied and is not well understood. Most of the reports antedate the recent increased recognition of the clinical significance of anaerobes and the availability of good anaerobic bacteriological procedures. Early reports by Andrews and Henry (2) and Edlund et al. (5) described *Clostridium* and anaerobic cocci as being re-

covered from 4 to 9% of cultured bile from patients who had no evidence of active infection. Finegold (7) has recently reviewed studies of similar groups of patients. In six of these large groups (100 or more patients), anaerobes were recovered from 1 to 16% of cultures. Finegold also reviewed anaerobic biliary tract infections, but this group consists of reports of small numbers of cases; many are single case reports. *Clostridium*, anaerobic cocci, and *Bacteroides* were most commonly isolated. The most extensive survey to date included 1,000 consecutive cholecystectomies in which 419 bile cultures were obtained. The authors reported confirming only a single case of anaerobic infection (14). In view of this paucity of information from any one institution concerning the role of anaerobes in biliary tract infection, we present our experience at the Mayo Clinic over a 2-year period, during which optimum specimen collection, transport, isolation, and identification of anaerobes were carried out.

MATERIALS AND METHODS

During the 2-year study period (April 1974 to April 1976), 1,892 patients underwent biliary tract surgery at the Mayo Clinic. The most common procedure was a cholecystectomy and exploration of the common bile duct because of cholecystitis and cholelithiasis. In most of the remaining cases, the gallbladder had been removed at a prior operation, and common duct exploration for choledocholithiasis was performed. A total of 562 patients had cultures, but only 371 of these had both aerobic and anaerobic cultures performed. This latter group constituted our study pop-

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ulation (Table 1). No surgeons routinely cultured the biliary tract. Criteria for obtaining cultures included clinical or gross pathological evidence of cholecystitis or cholangitis, preoperative fever with no source outside the biliary tract, and the presence of extrahepatic bile duct obstruction. Some patients received antimicrobial therapy (1 to 2 days) or prophylaxis (1 to 2 h) before surgery.

Bile was obtained by needle and syringe aspiration from the gallbladder or common bile duct during surgery in 75% of patients. The remainder of specimens were from T-tube drainage. The bile was then injected into a commercially available prereduced anaerobic transporter (Grand Island Biological Co., Grand Island, N.Y.). The techniques used for isolation and identification of both anaerobes (18) and aerobes (22) have been described in previous publications from the Mayo Clinic Laboratory. The records of this laboratory were the source of bacteriological data, and each patient's clinical record was reviewed by one of the authors.

RESULTS

The results of biliary tract cultures are listed in Table 1. Two hundred fifty-three patients, or 68% of those who were cultured for both aerobes and anaerobes, had positive cultures. These provided a total of 286 positive cultures, which was 69% of the total of 414 aerobic and anaerobic cultures performed. One hundred nineteen cultures yielded anaerobic bacteria, which represented 29% of the total aerobic and anaerobic cultures obtained or 42% of the total of positive cultures. Table 2 analyzes the 286 positive biliary

TABLE 1. *Biliary tract cultures, 1974-1976*

	No.	%
Total patients having cultures	562	30 ^a
Patients with both aerobic and anaerobic cultures	371	20 ^a
Patients with positive cultures	253 ^b	68 ^b
Total aerobic and anaerobic cultures	414 ^b	
Positive cultures	286	69 ^c
Cultures positive for anaerobes	119	29 ^c (42 ^d)

^a Based on total of 1,892 biliary tract operations.

^b From 371 patients.

^c Based on total of 414 cultures.

^d Based on total of 286 positive cultures.

tract cultures that were obtained from 253 patients. Aerobic bacteria only (i.e., no anaerobes present) grew from 167 cultures obtained from 153 patients, or approximately 60% of the totals. Most commonly, only one organism was isolated per culture; however, some cultures contained as many as six isolates. Anaerobic bacteria were isolated from the remaining 119 cultures obtained from 100 patients. However, in only two of these were anaerobes present in pure culture. Thus, 117 or 41% of the total positive cultures obtained from 39% of the patients contained a mixed flora of aerobes and anaerobes. These cultures most commonly contained four different organisms (three aerobes and one anaerobe), but some cultures yielded as many as nine. It should also be noted that in only 28% of the patients was a pure culture of a single bacterium found; the remainder were polymicrobial, containing mixtures of aerobic or aerobic and anaerobic bacteria.

The specific bacteria isolated from 286 positive bile cultures from the 253 patients are shown in Tables 3 and 4. A total of 858 isolates were recovered, of which 701 (82%) were aerobes and 157 (18%) were anaerobes. In all, 33 different species of aerobes and 26 different species of anaerobes were identified. Almost one-half (46%) of the aerobes were members of the family *Enterobacteriaceae* and, together with other gram-negative bacilli, accounted for 63% of all isolates. *E. coli*, the most common organism, represented 24% of the aerobic isolates. Group D streptococci (19%) and *Klebsiella* (17%) were the next most commonly isolated aerobes. *Pseudomonas aeruginosa* and the viridans streptococci provided 8 and 7% of the isolates, respectively, and each of the following organisms accounted for approximately 3%: *Enterobacter cloacae*, *Proteus mirabilis*, *S. epidermidis*, and *Proteus morganii*. The remaining organisms, in decreasing frequency of isolation, are listed in Table 3.

Anaerobic gram-negative bacilli and *Clostridium* accounted for 89% of all anaerobic bacteria isolated from biliary tract cultures (Table 4). Members of the genus *Bacteroides* were most

TABLE 2. *Results of 286 positive biliary tract cultures obtained from 253 patients*

Isolates	No. of patients (%)	No. of cultures (%)	No. of isolates	Range ^a	Mode ^b
Aerobes only	153 (60)	167 (58)	330	1-6	1
Anaerobes only	2 (1)	2 (1)	2	1	1
Mixed aerobes and anaerobes	98 (39)	117 (41)	528	2-9	4
Aerobes	98	117	371	1-7	3
Anaerobes	98	117	157	1-4	1

^a Range of different isolates per culture.

^b Mode values of different isolates per culture.

TABLE 3. *Aerobic bacteria isolated from biliary tract cultures*^a

Organism	No. of isolates ^b
<i>Escherichia coli</i>	169 (24)
<i>Streptococcus</i> , group D	132 (19)
<i>Klebsiella</i>	116 (17)
<i>Pseudomonas aeruginosa</i>	56 (8)
<i>Streptococci</i> , viridans group	46 (7)
<i>Enterobacter cloacae</i>	24 (3)
<i>Proteus mirabilis</i>	23 (3)
<i>Staphylococcus epidermidis</i>	22 (3)
<i>Proteus morganii</i>	21 (3)
<i>Citrobacter freundii</i>	14 (2)
<i>Corynebacterium</i> species	12 (2)
<i>Pseudomonas</i> species	9 (1)
<i>Enterobacter aerogenes</i>	8 (1)
<i>Staphylococcus aureus</i>	7 (1)
<i>Citrobacter diversus</i>	6 (1)
<i>Lactobacillus</i>	6 (1)
Miscellaneous	30 ^c

^a Total of 284 cultures obtained from 251 patients, including 98 mixed infections and 153 with aerobes only isolated.

^b Numbers in parentheses are percentage of total of 701 isolates.

^c Includes species of *Acinetobacter*, *Aerococcus*, *Aeromonas*, *Alcaligenes*, *Bacillus*, *Eikenella*, *Enterobacter*, *Proteus*, *Serratia*, and *Streptococcus* (non-group D).

commonly recovered; 71 organisms grew from 65 cultures obtained from 59 patients and provided 45% of the anaerobic isolates. Clostridia were next in order of frequency (39%). *Bacteroides fragilis* was the single most commonly isolated anaerobe, accounting for 38% of isolates, and next in frequency was *Clostridium perfringens* (33%). All other anaerobes were isolated much less frequently, with nonsporing gram-positive bacilli, *Fusobacterium*, and anaerobic cocci being recovered only 8, 5, and 3% of the time, respectively. The individual anaerobic species are listed in Table 4. In terms of overall frequency of isolation, *B. fragilis* ranked fourth behind *E. coli*, group D streptococci, and *Klebsiella*; it accounted for 7.0% of the total aerobic and anaerobic isolates (858) and was present in 21% of all positive cultures. *P. aeruginosa* and *C. perfringens* ranked fifth and sixth, providing 6.5 and 5.9% of all isolates, respectively.

Pure cultures of anaerobes were grown from only two patients, and these were *C. perfringens* in one case and *Fusobacterium* species in the other. In addition, four mixed aerobic and anaerobic cultures contained more anaerobes than aerobes. Isolates of *Bacteroides* were the only anaerobes present in 59% of patients with mixed cultures. In the remaining 41% of patients with mixed cultures, more than one anaerobic genus

was present, and the most frequent combination was *Bacteroides* and *Clostridium*. Mixed cultures from seven patients contained three anaerobes, and an additional one contained four different anaerobes (*B. fragilis*, *Bacteroides* species, *C. perfringens*, and *Fusobacterium* species) in addition to the aerobic isolates.

The incidence of various aerobes and anaerobes in T-tube cultures and bile obtained at surgery was similar. In six of a total of eight patients who had both T-tube and surgical cultures performed, the results were essentially identical.

DISCUSSION

This bacteriological study of bile in patients with evidence of active biliary tract infection or bile duct obstruction demonstrates the frequent presence of anaerobic bacteria in this setting. This fact is not appreciated by some infectious disease clinicians who exclude the biliary tract in their consideration of anaerobes in abdominal infections (3). Anaerobes were recovered from approximately 40% of our patients with bactibilia, and *B. fragilis*, the most common anaerobe,

TABLE 4. *Anaerobic bacteria isolated from biliary tract cultures*^a

Organism	No. of isolates ^b
Anaerobic gram-negative bacilli	78 (50)
<i>Bacteroides</i> ^c	71 (45)
<i>B. fragilis</i>	60 (38)
<i>Fusobacterium</i> ^d	7 (5)
<i>Clostridium</i> ^e	61 (39)
<i>C. perfringens</i>	51 (32)
Nonsporing gram-positive bacilli ^f	13 (8)
Anaerobic cocci ^g	5 (3)

^a Total of 119 cultures obtained from 100 patients, including 98 mixed infections and 2 with anaerobes only isolated.

^b Numbers in parentheses are percentage of total of 157 isolates.

^c Includes isolates of *B. fragilis*, *B. melaninogenicus* (6), *B. ruminicola* (1), *B. clostridiiformis* (1), and *Bacteroides* species (3).

^d Includes isolates of *F. nucleatum* (2), *F. mortiferum* (1), *F. necrophorum* (1), and *Fusobacterium* species (3).

^e Includes isolates of *C. perfringens*, *C. ramosum* (2), *C. difficile* (1), *C. subterminale* (1), *C. tyrobutyricum* (1), and *Clostridium* species (5).

^f Includes isolates of *Propionibacterium acnes* (4), *Bifidobacterium adolescentis* (2), *Bifidobacterium ericksonii* (1), *Bifidobacterium* species (1), *Eubacterium* species (1), and nonidentified nonsporing gram-positive bacillus (4).

^g Includes isolates of *Peptostreptococcus anaerobius* (1), *P. intermedius* (1), *P. micros* (1), *P. prevotii* (1), and *Veillonella* (1).

was exceeded in frequency of isolation only by *E. coli*, group D streptococci, and *Klebsiella*. Species of *Bacteroides* and *Clostridium* accounted for 84% of anaerobic isolates and were present almost exclusively in mixed cultures with aerobes.

We believe our study is unique in comparison with previous reports because of the large number of patients who had cultures for both aerobes and anaerobes (371), the large number of positive cultures (286), and our use of modern, optimal methods for transport, isolation, and identification of anaerobes. Our results differ from those of most previous reports in that we found a higher frequency of anaerobic isolations and that *Bacteroides* (especially *B. fragilis*), not *Clostridium*, was the most commonly encountered anaerobic genus. We agree with the recent report of Shimada et al. (21), who found that *B. fragilis* was the most frequent anaerobic isolate from bile and ranked third behind *E. coli* and *Klebsiella-Enterobacter* in overall isolation rate. However, their study included only 40 positive cultures, and all were from elderly Japanese nursing-home patients who had a high incidence of pigmented gallstones. Our results are certainly more representative of the overall spectrum of a general group of North American patients with biliary tract disease. Another interesting observation from our data is the rather high recovery rate of *P. aeruginosa* (fourth most common aerobic isolate and fifth most common overall). Most other studies report lower isolation rates for this organism (11, 15, 21).

As Shimada et al. (21) have suggested, the increased isolation of *Bacteroides* in these studies is probably related more to improvements in anaerobic laboratory methods than to any real change in their incidence in biliary tract disease. Clostridia are more aerotolerant than most other anaerobes and are more likely to have grown in the less efficient anaerobic isolation systems used in previous years.

The mechanism by which anaerobes populate a diseased biliary tract is unknown, although the organisms almost certainly originate in the gastrointestinal tract and either ascend directly via the common bile duct or are transported in the portal circulation, as has been suggested by animal experiments (13). Gallstones and ductal obstruction seem to be predisposing circumstances (12, 19).

This study does not establish the clinical significance of anaerobic bactibilia—this will be the subject of a future report. However, the frequent presence of anaerobes (especially *B. fragilis*) in this area should lead the clinician to consider these organisms in formulating an

antimicrobial regimen for treatment of biliary tract sepsis. Unfortunately, anaerobic infection may be difficult to confirm without surgically obtained cultures because anaerobic bacteremia originating from the biliary tract is uncommon. Only 3% of our patients with mixed cultures had positive blood cultures containing anaerobes (10% of 31 bacteremic patients). Shimada et al. (21) noted a similar low incidence of anaerobic bacteremia. Nevertheless, when sepsis originates from the biliary tract, anaerobes should be considered as possible infecting pathogens. In particular, our clinical experience is similar to that of others who have reported the frequent occurrence of anaerobic wound infections when bactibilia has been discovered at surgery, especially when bile duct obstruction is also present (6, 11, 12, 21).

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