Aerobic and Anaerobic Microbiology of Biliary Tract Disease

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A retrospective analysis of the experiences of two military hospitals over 4 years in the recovery of organisms from biliary tract specimens was done. Bacterial growth was obtained in 123 bile specimens. Aerobic and facultative bacteria only were present in 59 specimens (48%), aerobic bacteria only were present in 4 specimens (3%), and mixed anaerobic and aerobic or facultative bacteria were present in 60 specimens (49%). Of 286 isolates recovered, 216 were aerobic or facultative (1.8 per specimen) and 70 were anaerobic (0.6 per specimen). The predominant bacteria were Escherichia coli (71 isolates), group D streptococci (42 isolates), Klebsiella sp. (29 isolates), Clostridium sp. (27 isolates), Bacteroides sp. (28 isolates), and Enterobacter sp. (16 isolates). Polybacterial infections were present in 108 instances (88%). A higher recovery rate of anaerobes was present in patients with chronic infections than in those with acute infections and did not correlate with the presence of gallstones or use of antimicrobial prophylaxis.

Awareness of the role of anaerobic bacteria in biliary tract disease has increased in recent years. These organisms have been recovered in about one-half of patients with cholangitis whenever techniques for their recovery have been used (1, 8, 12, 13, 15). However, only a few studies have defined predisposing conditions and the clinical features characteristic of anaerobic infections of the biliary tract (1, 13, 15). Furthermore, the strains of anaerobic bacteria have not been defined at the species level in many of these studies (1, 8, 12, 13, 15). Since the publication of most of these reports, the methods of collection, transportation, cultivation, and identification of anaerobes have improved. Furthermore, many of the anaerobes have been reclassified and renamed by newer criteria. Therefore, a more current evaluation of the incidence of recovery of anaerobes in biliary specimens is desirable. This report summarizes the experiences of the microbiology laboratories in two military hospitals during a 4-year period in the recovery of anaerobic bacteria from biliary tract specimens. The data provide in detail the recovery rates of all anaerobes, including some of the newly reclassified species.

Microbiological data on biliary tract specimens processed for aerobic and anaerobic bacteria in the clinical microbiology laboratories at Walter Reed Army Medical Center in Washington, D.C., and the Naval Hospital in Bethesda, Md., between June 1981 and June 1985 were reviewed. Excluded from evaluation were specimens obtained from patients who received any antimicrobial therapy (except prophylaxis) before surgery.

A prophylactic antibiotic was administered to 117 patients (81%), 50 with chronic disease and 48 with acute inflammation. The anaerobic agent used for prophylaxis was either a narrow- or extended-spectrum cephalosporin, and this was administered once before and twice after surgery. Of the 145 specimens that were included in the final analysis, bacterial growth was detected in 123 (85%). All of these specimens were obtained from patients who underwent elective or emergency biliary tract surgery. The mean age of the patients was 53 years (range, 15 to 84), and 95 were males. The cases were grouped by diagnosis as follows: cholecystitis, 32 cases; cholecystitis with gallstone(s), 82 cases; carcinoma of the biliary tract, 6 cases (4 with gallstones); benign stricture of the common bile duct, 3 cases. Cholecystectomy was performed in 104 patients, and gallbladder specimens were examined histologically. Of the 104 gallbladders, 55 showed chronic inflammation and the others showed acute inflammation (15).

Samples of gallbladder bile were aspirated aseptically by syringe during the operation, sent to the laboratory in a syringe with a rubber stopper on the needle, and plated within 30 min of collection. Whenever a longer delay was expected, the specimens were placed in anaerobic transport medium (Port-A-Cul vial; BBL Microbiology Systems, Cockeysville, Md.). The Port-A-Cul system recovers as many anaerobic bacteria during prolonged transport as would be recovered by a freshly cultured specimen (5).

Specimens were inoculated onto 5% sheep blood, chocolate, and MacConkey agar plates for aerobic and facultative organisms. The plates were incubated aerobically at 37°C (MacConkey agar) or under 5% CO2 (5% sheep blood and chocolate agars) and examined at 24 and 48 h. For anaerobes, the material was plated onto prereduced vitamin K1-enriched bruccella blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin, and an anaerobic blood plate containing colistin and nalidixic acid and inoculated into enriched thioglycolate broth (containing hemin and vitamin K2) (16). The anaerobic plates and thioglycolate broth were incubated in GasPak jars (BBL) and examined at 48 and 96 h.

Anaerobes were identified by techniques described previously (16). The Clostridium botulinum isolate was identified by the Centers for Disease Control (Atlanta, Ga.) laboratories. Aerobes were identified by conventional methods (10). Clinical and historical information was correlated with microbiological data. Statistical analysis was done with the Student t test.

Aerobic or facultative bacteria only were recovered in 59 specimens (48%), anaerobic bacteria only were recovered in 4 specimens (3%), and mixed anaerobic and aerobic or facultative bacteria were recovered in 60 specimens (49%). A total of 286 isolates (2.4 per specimen) were recovered; 216 were aerobic or facultative (1.8 per specimen), and 70 were anaerobic (0.6 per specimen). The predominant aerobic or facultative organisms were Escherichia coli (71 isolates),

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group D streptococci (42 isolates), Klebsiella sp. (29 isolates), and Enterobacter sp. (26 isolates) (Table 1). The predominant anaerobic bacteria were Bacteroides sp. (28 isolates, including 22 B. fragilis group isolates) and Clostridium sp. (27 isolates, including 23 C. perfringens isolates).

Single isolates were recovered in 15 instances (12%). These included E. coli (eight isolates), Klebsiella sp. (three isolates), Enterobacter sp. (two isolates), and B. fragilis and C. perfringens (one isolate each). The rest of the specimens (108 or 88%) had polymicrobial flora of two to eight isolates per specimen.

The relationship between the type of bacteria and the pathological characteristics of acute and chronic inflammations shows a statistically significantly (P < 0.01) higher recovery rate of anaerobes in chronic inflammation (34 of 55 cases) than in acute inflammation (17 of 49 cases). Anaerobic bacteria were not isolated more frequently from patients with gallstones (44 of 82) than from those without stones (14 of 32). The use of antimicrobial agents for prophylaxis did not affect the recovery rate of organisms.

Like other investigations (8, 13, 15), this study confirms the recovery of anaerobes (especially B. fragilis group and C. perfringens) in over 50% of patients with inflammatory bile disease. The present study shows a correlation between chronicity of inflammation and recovery of anaerobes. Anaerobes were recovered in 35% of acutely inflamed and in 62% of chronically inflamed biliary systems. The predominance of anaerobes in chronic inflammation was also noted by Marne et al. (13) but not by Bourgaut et al. (1).

Anaerobes are more commonly isolated in chronic than acute infections in a variety of body sites (2, 6). It is postulated that the lowering of oxygen tension and pH achieved by aerobic bacteria in the acute stages of polymicrobial infection paves the way for the predominance of anaerobes in the chronic stage of illness (14). The emergence of a capsule, an important virulence factor of Bacteroides sp. and Peptostreptococcus sp. (4), which requires 7 to 10 days, also explains the predominance of these organisms in the later stages of infection.

The polymicrobial nature of chronic biliary infection is evident. Polymicrobial infections are known to be more pathogenic for experimental animals than are infections involving single organisms (7). Synergy between E. coli, the most commonly recovered aerobic isolate, and the B. fragilis group, the most common anaerobic isolate, has been demonstrated in an in vivo abscess model (3) and in a biliary tract infection model using rabbits (11). The recovery of anaerobes in over 50% of the patients is not surprising, since these bacteria colonize the gastrointestinal tract (9) and can reach the biliary system directly or through the lymphatics.

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**LITERATURE CITED**