

Microbiological Investigation with Bile Cultures in Cholecystectomy Cases[†]

KOLESİSTEKTOMİ OLGULARINDA SAFRA KÜLTÜRLERİNİN MİKROBİYOLOJİK ANALİZİ

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Summary

Purpose:The aim of this study was to identify the relationship of bacterial growth in the cultures of patients undergone open cholecystectomy with the age of patients, clinical diagnosis, histopathological diagnosis and prophylactic antibiotherapy.

Method:Bile samples were obtained peroperatively from 109 patients and then a prospective assessment of antibiotic sensitivity tests, clinical status of the patients and histopathological findings was made.

Results: Ninety-one out of 109 patients were female, 18 were male (M/F=1/5). The mean age was 52.4. The most common indication for cholecystectomy was chronic calculous cholecystitis (83.5%). A positive bile culture was found in 19.3% of all the cases. Bile culture positivity was highest in the patients with obstructive jaundice (66.7%). There wasn't any bile culture positivity in chronic acalculous cholecystitis cases. Chronic cholecystitis was the most observed histopathological diagnosis (78.9%). Among the age groups, the highest bacterial growth rate was observed in patients with the age of 70 and over (38.5%). The most common isolated bacteria was E.Coli (30.4%). When used for prophylactic measures, there was no significant difference between cefazolin and ampicillin/sulbactam in terms of efficacy on the bile cultures.

Conclusion: In view of this study's findings which are similar to the results of other studies, we suggest that prophylactic antibiotic coverage is necessary in open cholecystectomy cases, especially when the patients is old, has calculous cholecystitis or any additional gallbladder pathology.

Key Words: Cholecystectomy, Bacterial Growth

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Özet

Amaç: Açık kolesistektomi olgularında yapılan safra kültürlerinde bakteriyel üremenin hastanın yaşı, kliniği, histopatolojik tanı ve profilaktik antibiyotik kullanımı ile ilişkisini ortaya koymayı amaçladık.

Metod: Kolesistektomi uygulanan 109 hastadan peroperatuvar safra kültürleri alınarak kültür-antibiyoqramları ile klinik ve histopatolojik bulguları prospektif olarak incelendi.

Bulgular: 109 hastadan 91'i kadın, 18'i erkekti (E/K=1/5). Yaş ortalaması 52.4 dü. Çalışmada kolesistektominin en sık endikasyonu kronik taşlı kolesistit idi (%83.5). Tüm olgularda üreme oranı %19.3 idi. Tıkanma ikteri olgularında safra kültürlerinde üreme oranı en yüksekti (%66.7). Kronik taşsız kolesistitlerde ise safra kültürlerinde hiç üreme olmadı. En sık görülen histopatolojik tanı kronik kolesistit oldu (%78.9).Yaş gurupları arasında en yüksek üreme oranı 70 yaş ve üzerinde bulundu(%38.5).Kültürlerde en sık izole edilen bakteri E.Coli oldu (%30.4). Profilaksi amacıyla kullandığımız Sefazolin ve sulbaktam/ampisilin antibiyotiklerinin safra kültürleri üzerindeki etkinlikleri arasında anlamlı bir farklılık bulunamadı.

Sonuç:Diğer araştırmaların sonuçları ile benzerlik arzeden bu çalışmanın sonuçları açık kolesistektomi geçirecek olan özellikle yaşlı ve ek safra yolları patolojisi olan taşlı kolesistit olgularında profilaktik antibiyotik kullanımının gerekli bir uygulama olduğunu göstermiştir.

Anahtar Kelimeler: Kolesistektomi, Bakteri Kültürü

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There are many studies reporting the relationship between the existence of microorganisms in the gallbladder and postoperative wound infection

(1-3). Infectious complications such as septicemia, cholangitis and liver abscess are also seen more frequently when the bile culture is positive (4). It

has been reported that the risk for bacterial growth in bile cultures was higher in patients having acute cholecystitis, cholangitis, obstructive jaundice, diabetes mellitus or when the patient was aged over 60 (5-8). Our main objective was to define the microorganisms causing bile duct infection and their distribution according to age, sexuality and clinical diagnosis. Another objective was to compare the effects of two different antibiotics in respect of bacterial growth on microbiological culture. We also aimed to identify whether there is a relation between bacterial growth rate and pathological diagnosis.

Patients and Methods

One hundred and twelve patients who underwent open cholecystectomy operation in Ministry of Health Ankara Research and Training Hospital Second and Third General Surgery Departments between February 1997 and March 1999 were enrolled in the study. Three patients were excluded because of the contamination in the bacterial cultures. Finally, 109 patients were taken into the study.

In this study, bile samples were obtained peroperatively from the patients who underwent open cholecystectomy. A single dose of antibiotic was administered to each patient one hour before the operation for the purpose of surgical prophylaxis. This antibiotic was either cefazolin (1st generation cephalosporin 1g, i.v.) in 67 patients or ampicillin/sulbactam (1 g, i.v.) in 42 patients. As soon as having the gallbladder excised, 3-4 ml bile aspirate was collected with a sterile syringe. Samples were transported to the microbiology laboratory immediately after they were collected. Aerobic culture of the isolates, bacteriological identification and antibiotic sensitivity tests were done. Anaerobic cultures were not done because practicing anaerobic cultures was difficult. Besides, anaerobic factors related to the gallbladder were seldomly reported in the literature.

Age, sexuality and clinical diagnosis of the patients, antibiotics used for prophylaxis, results of the cultures and antibiotic sensitivity tests and histopathological diagnosis of the surgical materi-

als were all recorded. Statistical analyses were performed with chi-square test (Pearson's).

Microbiological Study

Samples obtained were first cultured on thio-glucanate medium. They were conveyed onto chocolate agar and blood agar after the turbidity was seen. After 24 hours of incubation period, they were evaluated. Catalase test was applied to Gram-positive microorganisms in order to differentiate *Staphylococcus* and *Streptococcus spp.* Coagulase test was performed for *Staphylococcus aureus*. Bacitracin, optokin and strep-latex tests were performed for *Streptococci*. Oxidase test was applied for Gram-negative organisms. Samples were cultivated onto TSI agar, citrate agar, phenyl-alanine agar, Voges Proskauer agar, indole methyl red, lysin and ornithine decarboxylase agar. Then the cultures were incubated for 24 hours.

Results

One hundred and nine patients were included in the study. Ninety-one (83.5%) of the patients were women while 18 (16.5%) were men. The mean age of the patients was 52.4 (ranging from 19 to 75). Mean age of the women was 52.2, mean age of the men was 53.7.

The most common indication for cholecystectomy was chronic calculous cholecystitis (91 patients, 83.5%) followed by acute cholecystitis (12 patients, 11%) and chronic acalculous cholecystitis (3 patients, 2.8%) and obstructive jaundice (3 patients, 2.8%). Seven of the acute cholecystitis cases were of the calculous kind.

Bile culture was positive in 21 patients (19.3%). Number of the microorganisms isolated was 23. Bile culture positivity was highest in the patients aged 70 and over (38.5%). Then 20-29 age group followed (27.3%). The positivity was lowest in 50-59 age group (9.4%). But these differences were not statistically significant ($p > 0.05$).

Bile culture positivity rate was highest in the obstructive jaundice group. There were 3 patients in this group and bile cultures were positive in two of them (66.7%). There was no bacterial growth in

Table 1. Bile culture positivity rates according to clinical diagnosis

Clinic Diagnosis	n	Bile culture positivity	Percentage (%)	P
Chronic Calculous Cholecystitis	91	16	17.6	NS
Acute Cholecystitis	12	3	25	NS
Obstructive Jaundice	3	2	66.7	NS
Chr. Acalculous Cholecystitis	3	0	0	NS
Total	109	21	19.3	

NS:Not significant

chronic acalculous cholecystitis group. Bile culture positivity rates according to clinical diagnosis are shown in Table 1.

The microorganisms isolated from bile samples were classified according to their gram staining characteristics. Fourteen of them were Gram-negative, 8 of them were Gram-positive and 1 of them was *Candida albicans*. Fourteen strains which were isolated in chronic calculous cholecystitis cases were: 8 *Gram-negative enteric bacteria*, 5 *Gram-positive cocci*, 1 *Candida Albicans*. All of the growing bacteria isolated in acute cholecystitis cases were *Gram-negative enteric bacteria*. In obstructive jaundice, *E.Coli* and *Enterococci* were isolated. The most commonly isolated bacteria was *E. coli* (7, 30.4%) followed by *Salmonella paratyphi* (3,13%), *coagulase (-) Staphilococcus spp.* (3, 13%), *Enterococcus* (2, 8.6%), *Nonhemolytic streptococcus* (2, 8.6%) and *Klebsiella oksitake* (2, 8.6%). The following bacteria, *Salmonella typhi*, *Staph aureus*, *Klebsiella pneumoniae* and *Candida albicans* were each isolated in only one culture (1, 4.3%).

The results of the antibiotic susceptibility tests were as follows: All of the Gram-negative strains (14 cases) were susceptible to ceftriaxone, amikacin and imipenem. There were 7 strains which were susceptible to cefazolin and ampicillin/sulbactam, the antimicrobial agents used for surgical prophylaxis in this study. All of the Gram-positive bacteria were susceptible to vancomycin. The number of strains susceptible to cefazolin was 4. This number was 3 for ampicillin/sulbactam.

Bacterial growth was positive in 13 of the 67 patients for whom cefazolin had been used for

surgical prophylaxis (19.4%). Bacterial growth was noted in 8 of 42 patients (19%) for whom ampicillin/sulbactam had been used for surgical prophylaxis. The difference between these two antibiotics was not statistically significant ($p>0.05$).

The results of the histopathological examination revealed that the most common pathological diagnosis was chronic cholecystitis (86, 78.9%). Chronic cholecystitis +cholesterolosis group followed this group (14, 12.8%). The least common pathological diagnosis was chronic cholecystitis with acute exacerbation (9, 8.3%). All the obstructive jaundice cases were in this histopathological group.

In chronic calculous cholecystitis group 9 patients had additional gallbladder pathology. These were hydropic gallbladder (3 patients), choledocholithiasis (3 patients), polyp (1 patient), gallbladder carcinoma (1 patient), liver hydatid cyst (1 patient). In acute cholecystitis group, 5 patients had some complications related to infection; these were empyema (2 patients), cholangitis (2 patients) and closed perforation (1 patient).

Bile culture positivity rate according to histopathological diagnosis are shown in Table 2. Bacterial growth was highest in chronic cholecystitis+cholesterolosis group (28.6%); the lowest rate was recorded in chronic cholecystitis group (17.4%). But these differences were not statistically significant ($p>0.05$).

Discussion

Bile culture positivity rates vary in different studies. This variety may be due to the difference in the proportion of acute and chronic cholecystitis

Table 2. Bile culture positivity rates according to histopathological diagnosis

Histopathologic Diagnosis	n	Bile Culture Positivity	Percentage (%)	p
Chronic Cholecystitis	86	15	17.4	NS
Chronic Cholecystitis+Cholesterolosis	14	4	28.6	NS
Acute Exacerbated Chr.Cholecystitis	9	2	22.2	NS
Total	109	21	19.3	

NS: Not significant

cases in different studies. In our study, bile culture positivity rate was found to be 21 in 109 patients (19.3 %). In the two previous studies accomplished in our country, this rate was found to be 27% and 36% (9,10).

Bile culture positivity rate is generally reported to be high in patients with acute cholecystitis, obstructive jaundice, diabetes mellitus and in immun-compromised patients and patients over 60 years of age (1,2,5-8,11-13). According to Al Harbi et al. Reported that bile culture positivity was significantly higher in patients over the age of 50 (14). In our study, bacterial growth rate was found to be 38.5% in the patients over 70 years. All of the bacteria isolated in this age group were Gram-negative enteric bacteria and enterococcus. Besides, *Candida albicans* was isolated in one patient in this group. This was an expected finding. Because *Candida albicans* is known to be found in older age group and immunocompromised patients (15,16).

Bacterial growth rate in the bile samples of patients with chronic calculous cholecystitis was 17.1% (14 in 82 patients). This rate was 28.6% for acute cholecystitis patients (2 in 7 patients). Bacterial growth was found in two of the three patients with obstructive jaundice (66.7%). This finding supports the fact that stasis in gall bladder is associated with increased bacterial growth rates. There was no growth in chronic acalculous cholecystitis group. Müslümanoğlu reported that in acute cholecystectomy cases bile culture positivity rate was 100% (17). Growth rate was in our study slightly higher in patients with chronic cholecystitis and additional pathology (polyp, choledocholithiasis, hydropic gallbladder, malignancy, hydatid cyst) compared with that of the patients with acute complicated cholecystitis (perforation, acute cholangitis,

empyema). But this difference was not statistically significant ($p>0.05$). The explanation for this can be that additional pathologies increase the risk of passage of enteric bacteria to biliary tract (11,18).

Escherichia coli was the most frequently isolated organism in our study (30.4% of isolates). *Salmonella paratyphi* and coagulase (-) *Staph spp.* follow *E. coli* (13%). *E. coli* was reported to be the most frequently isolated bacteria in the previous studies (9,17). When we classified the isolated microorganisms according to their microbiological characteristics, the most frequently isolated organisms were gram (-) enteric bacteria. Gram (+) cocci came thereafter. Al Harbi et al. Reported that there weren't any anaerob bacterias detected in their study (14).

Antibiotic sensitivity test results suggested that gram (-) microorganisms isolated from bile were mostly sensitive to ceftriaxon, amikacin and imipenem, while gram (+) microorganisms were mostly sensitive to vancomycin, penicillin and chloramphenicol. There wasn't considerable resistance against any of the antibiotics used in antibiotic sensitivity tests.

Bacterial growth rates were nearly the same (19.4% vs.19%) for both cefazolin and ampicillin / sulbactam groups. A very low concentrations of cefazolin and sulbactam /ampicillin were also seen in the bile samples taken during the operations whereas blood and wound concentrations of both antibiotics reached at adequate levels (19). Therefore we conclude that the effect of antibiotics on our bile cultures can be considered as minimal.

Pathologic examination revealed that bacterial growth was maximum in chronic cholecystitis+cholesterolosis group (28.6%). This value was 22.2% in chronic cholecystitis + acute exacerbation group. We had expected the highest growth

rate to be in chronic cholecystitis & acute exacerbation group.

This study indicated that gallbladder pathologies were more common in females than males in nearly all age groups (M/F=1/5). The possibility of microbiological invasion increased with increasing age. Bacterial growth rate was highest in patients at the age of 70 and over (38.5%). The most common indication for cholecystectomy was chronic calculous cholecystitis (75.1%). The rarest indication for cholecystectomy is chronic acalculous cholecystitis (2.8%). We isolated bacteria in 19.3% of the cases. Bile culture growth rate was highest in obstructive jaundice patients (66.7%). The most common isolated organism is *E. coli* (30.4%). The most common pathological diagnosis was chronic cholecystitis (78.9 %). Gram (-) microorganisms were mostly sensitive to ceftriaxon, amikacin and imipenem. Gram (+) microorganism are mostly sensitive to vancomycin and penicillin. The isolated microorganisms were moderately sensitive to the antibiotics we used for surgical prophylaxis. But this doesn't mean that ceftriaxon, amikacin, imipenem and vancomycin should routinely be used for surgical prophylaxis of cholecystectomy operations. Because they are all broad spectrum antibiotics, they should be reserved for the cases in which septic complications are suspected. In the light of our study we conclude that prophylactic antibiotic coverage is necessary in open cholecystectomy cases, especially when the patient is old, has calculous cholecystitis or any additional gallbladder pathology.

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