Microorganisms isolated from the bile of the patients who have undergone cholecystectomy and their antibiotic resistance pattern: multicenter prospective study

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Abstract

Background Gallbladder and biliary tract infections are diseases with high mortality rates if they are not treated properly. Microbiological evaluation of perioperatively collected samples both ensures proper treatment of patients and guides empirical treatment due to the determination of microorganism susceptibility.

Aims This study aimed to isolate the microorganisms in bile cultures from patients who underwent cholecystectomy and to determine sensitivity results of these microorganisms.

Methods This study was a multi-center and prospective design, included 360 patients, and was performed between 2019 and 2020. Culture results of bile taken during cholecystectomy were evaluated.

Results Bacterial growth was found in the bile cultures of 84 out of 360 (23.3%) patients. Patients were divided into two groups according to whether they had risk factors for resistant microorganisms or not. While Escherichia coli (n = 11, 13%), Enterococcus spp. (n = 8, 9.5%), and Enterobacter spp. (n = 4, 4.7%) were detected most frequently in patients without risk. Staphylococcus spp. (n = 17, 20.2%), Enterococcus spp. (n = 16, 19%), and E. coli (n = 8, 9.5%) were the most frequently found microorganism at-risk patients. In multivariate analysis, bile culture positivity was found higher in patients who had history of biliary disease (p = 0.004), operation performed concurrently with a cholecystectomy (p = 0.035), and high rate of polymorphonuclear leukocytes (PNL) in total leukocyte count (p = 0.001).

Conclusions Our study shows that when starting empirical antibiotic treatment for bile ducts, whether patients are at risk for the development of resistant bacterial infection should be evaluated after which antibiotic selection should be made accordingly.

Keywords Cholecystectomy · Bile culture · Antimicrobial resistance

Introduction

Gallbladder and biliary tract infections are an important health problem. Obstruction of the bile flow is the most important cause of biliary tract infections. Obstruction causes an increase in pressure in the bile duct, and bacteria can reach the systemic circulation through hepatic sinusoids when there is high pressure (Gupta and Chakravarti 2008). Although the most important reason for obstruction of bile flow is gallstones, tumors originating from surrounding structures and surgical injury to the bile ducts can also cause obstruction in the biliary tract (Mustafa et al. 2014).

Some experts have stated that the presence of microorganisms in the bile does not always indicate an active infection (Park et al. 2014). However, the detection of bactobilia during biliary surgery not only serves as a guide for appropriate antibiotic prophylaxis but also for administration of empirical therapy and changes in antimicrobial therapy when necessary. Bactobilia is common in patients with risk
factors, such as advanced age, biliary obstruction, previous biliary instrumentation, acute cholecystitis, common bile duct stones, cholangitis and nonfunctioning gallbladder, previous biliary sepsis, bilioenteric anastomosis, and suppressed immune status (Maseda et al. 2013; Abeysuriya et al. 2008).

Gallbladder and biliary tract infections are diseases with high mortality rates if they are not treated properly. In patients with moderate or severe acute cholecystitis, antimicrobial therapy is recommended, and this therapy should be chosen considering the local antimicrobial resistance properties of microorganisms (Gomi et al. 2008). Limited information about the infectious agents in the bile ducts and their resistance patterns exists. This multicenter prospective study was designed to evaluate microorganisms with their corresponding resistance patterns that have been isolated from the bile of patients who have undergone cholecystectomy.

Methods

Study design

This multicenter and prospective study was performed in patients undergoing cholecystectomy between January 2019 and April 2020. Eight centers were included in the study. Consecutive patients were selected to participate in this study. The Ethical Committee of the Istanbul Fatih Sultan Mehmet Training and Research Hospital, Istanbul, Turkey, approved the study (FSMEAH-KAEK-2020/11), and informed consent was obtained from all participants.

Inclusion criteria

Patients aged >17 who were scheduled for cholecystectomy were included.

Data collection

Bile culture Recorded data included age, gender, body mass index (BMI), comorbidities, history of hospitalizations within the last year, biliary surgery history, history of biliary disease, presence of biliary instrumentation, history of antibiotic use in the last 3 months, symptoms of patients, American Society of Anesthesiologists (ASA) score, the presence of operation concurrently with cholecystectomy, complications, laboratory and culture results, and patterns of antimicrobial in the microorganisms.

During cholecystectomy, 5–10 ml of bile fluid was aspirated under sterile conditions and/or a 1.5-cm² biopsy sample was taken from the bile mucosa. The sample was placed in a sterile tube containing sterile saline. Bile fluid and mucosal samples were sent to the microbiology laboratory for cultivation on culture plates. Chocolate and MacConkey agars and aerobic and anaerobic blood culture media were used for cultures. The media were incubated at 35 to 37 °C. Aerobic media were kept for 18 to 24 and anaerobic media for 48 to 96 h. The counts of colonies that were above 10⁵ CFU/ml were considered significant. Growing strains were identified by conventional methods and VITEK 2® Compact (Biomerieux. France) and/or matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). VITEK-2® Compact automated systems were used to study antimicrobial susceptibility of microorganisms. The antibiogram data were analyzed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (EUCAST 2018).

Definitions

BMI was detected according to CDC. (https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html).

Risk factors Diabetes mellitus (DM), malignancy, immunosuppression, biliary disease, biliary surgery, biliary instrumentation, antibiotic use within the last three months, and/or hospitalization during the previous year were identified as the risk factors (Maseda et al. 2013; Iribar et al. 2017; Chow et al. 2010).

Acute cholecystitis Inflammation of the gallbladder with pain in the right upper quadrant (Adrian and Ian 2002).

MDR bacteria Acquired resistance to one antibiotic in at least three antibiotic classes (Magiorakos et al. 2012 Mar). Methicillin-resistant Staphylococcus spp., ampicillin-resistant Enterococcus spp., and extended spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria and carbapenem-resistant Gram-negative bacteria were assessed in this group.

Statistical analysis

The SPSS (version 23) program was used for statistical analysis. Descriptive statistics of the data are presented in tables as mean, standard deviation (SD), median, number, and % frequencies. The compliance of numerical data to the normal distribution was performed using the Shapiro–Wilk test. It was determined that numerical variables did not show normal distribution. The relationships between the presence of risk and other characteristics of the patients were evaluated using the Pearson’s chi-square analysis and the Mann–Whitney U test depending on the type and
distribution of variables. A $p$-value $<$ 0.05 was accepted as the statistical significance level. Variables that were found to be statistically significant in univariate tests were included in the multivariate binary logistic regression model, and their adjusted effects were calculated.

### Results

Gallbladder samples from 360 patients were taken during cholecystectomy. Most of the patients who participated in the study were women ($n=247$, 68.6%). Mean age was 51.1 ± 13.2 years. The duration of patients’ symptoms was 136.4 ± 212.2 days (median = 60; min–max = 1 and 1470).

Laparoscopic surgery was more frequently performed ($n=330$, 91.6%) compared to open surgery ($n=23$, 6.3%). An operation that started laparoscopically was converted to open surgery for seven patients (1.9%). Culture positivity was higher in patients with acute cholecystitis ($n=33/73$, 45.2%) than in patients with symptomatic cholelithiasis ($n=72/328$, 22.0%) ($p<0.001$).

Indications for cholecystectomy were symptomatic cholelithiasis in 328 patients (91.1%), acute cholecystitis in 71 patients (19.7%), acute pancreatitis in seven patients (1.9%), biliary polyps in three patients (0.8%), biliary and pancreatic malignant tumors, perforation in the gall bladder, and cytoreductive surgery in two patients (0.5%). Other indications were present in four patients (1.1%). Fifty-five patients (15.2%) underwent surgery for both symptomatic cholelithiasis and acute cholecystitis. Among the 71 patients with acute cholecystitis, 27 (38%) had biliary disease, five (7%) had a history of biliary surgery, and one (1.4%) had a history of biliary instrumentation and sixty-three (88.7%) patients underwent surgery while under treatment and eight (11.2%) patients underwent surgery with surgical prophylaxis.

One hundred sixty-five patients (45.8%) had underlying comorbid conditions. Hypertension and DM were the most common underlying conditions. Characteristics of the patients are presented in Table 1.

### Culture results

From a total of 84 patients (23.3%), 105 strains were grown. The most frequently isolated bacteria were Enterococcus spp. ($n=24$, 22.8%), Staphylococcus spp. ($n=22$, 20.9%), E. coli ($n=19$, 18.1%), Klebsiella spp. ($n=10$, 9.5%), Streptococci spp. ($n=8$, 7.6%), and Enterobacter spp. ($n=7$, 6.6%). Anaerobic culture was performed in 30% of the patients. There was no growth in the anaerobic culture.

Univariate analysis was performed in order to determine which factors affected bile culture positivity. Male gender ($p=0.001$), > 60 years of age ($p=0.01$), history of biliary disease ($p=0.002$), underwent biliary surgery before cholecystectomy ($p=0.002$), having biliary instrumentation ($p=0.014$), operation occurred concurrently with cholecystectomy ($p=0.001$), and/or presence of complications ($p=0.001$) were statistically significant. Because only two patients with a BMI < 19 were enrolled in the study, the BMI result was not considered significant (Table 2).

Leukocyte count ($p=0.017$), polymorphonuclear leukocyte (PNL) ratio ($p=0.001$), C-reactive protein (CRP) ($p=0.019$), creatinine ($p=0.008$), alkaline phosphatase (ALP) ($p=0.010$), total bilirubin ($p=0.003$), and glucose ($p=0.003$) values were found to be statistically significantly higher in patients with growth in the bile culture (Table 3).

According to multivariate analysis, bile culture positivity has been higher in patients with a history of biliary disease ($p=0.004$), operation performed concurrently with a cholecystectomy ($p=0.035$), and PNL percentage of > 64.9% in the total leukocyte count ($p=0.001$) (Table 4). Although conversion from laparoscopic to open cholecystectomy was a significant factor in the multivariate analysis, this was not considered significant due to small number of patients. Because only seven patients were converted to open surgery.

From a total of 26 patients, 31 multi-drug resistance (MDR) bacteria were identified. ESBL-producing Enterobacterales (ESBL-E) were detected in 25.6% among the Enterobacterales. Carbapenemase-producing

### Table 1 Clinic presentation of patients undergoing cholecystectomy ($n=360$)

| Underlying comorbid conditions       | Number | %  |
|--------------------------------------|--------|----|
| Hypertension                         | 80     | 22.2 |
| Diabetes mellitus                    | 55     | 15.2 |
| Ischemic heart disease               | 17     | 4.7 |
| Chronic obstructive pulmonary disease| 13     | 3.6 |
| Hypothyroidism                       | 11     | 3.1 |
| Malignancy                           | 9      | 2.5 |
| Heart rhythm disorder                | 9      | 2.5 |
| Congestive heart failure             | 6      | 1.6 |
| Chronic hepatitis B                  | 5      | 1.3 |
| Autoimmune disease                   | 5      | 1.3 |
| Chronic renal failure                | 3      | 0.8 |
| Hyperlipidemia                       | 3      | 0.8 |
| Peripheral vascular disease          | 2      | 0.5 |

| Symptoms and signs of the patients   | Number | %  |
|--------------------------------------|--------|----|
| Abdominal pain                       | 329    | 91.3 |
| Nausea-vomiting                      | 195    | 54.1 |
| Pain in the right shoulder            | 94     | 26.1 |
| Murphy sign                          | 36     | 10.0 |
| Fever                                | 33     | 9.1 |
| Back pain                            | 31     | 8.6 |
| Dyspeptic complaints                 | 17     | 4.7 |
Enterobacterales (CPE) were not detected. Carbapenem resistance was detected in one *P. aeruginosa* strain and one *A. baumannii* strain. Vancomycin-resistant *Enterococci* spp. was not isolated. Ampicillin resistance was found in nine enterococci strains. Methicillin resistance in *Staphylococci* spp. was detected in 54.5% of the isolates. Statistical analysis was performed to detect the risk factors associated with the isolation of MDR. Twenty-six patients who produced MDR and 58 patients who did not produce MDR were compared. No statistically significant result was found in univariate analysis (*p* > 0.05).

E. coli (*n* = 11, 1%), *Enterococcus* spp. (*n* = 8, 9.5%), and *Enterobacter* spp. (*n* = 4, 4.7%) were the most frequently found bacteria in patients without any risk factors. *Staphylococcus* spp. (*n* = 17, 20.2%), *Enterococcus* spp., (*n* = 16, 19%), and *E. coli* (*n* = 8, 9.5%) were the most frequently found bacteria at-risk patients.

In the no-risk patients, the resistance of cefuroxime axetil was determined as 67%; amoxicillin-clavulanate 46%; ceftriaxone, ceftazidime, and piperacilline-tazobactam 40% each; cefepime 27%, trimethoprim-sulfamethoxazole 20%, gentamycin 19%, and ciprofloxacin 15%. Imipenem-, meropenem-, and amikacin-resistant strains were not detected in these patients.

*E. coli* (*p* = 0.534), *Streptococcus* spp. (*p* = 0.644), *Citrobacter* spp. (*p* = 0.669), *Lactobacillus* spp.

| Table 2 The factors affecting culture positivity in the patients undergoing cholecystectomy | Culture negative | Culture positive | total |
|---|---|---|---|
| | n | % | n | % | n | p |
| >60 Age | No | 216 | 80.0 | 54 | 20.0 | 270 | 0.010 |
| Yes | 60 | 66.7 | 30 | 33.3 | 90 | |
| Gender | Male | 73 | 64.6 | 40 | 35.4 | 113 | 0.001 |
| Female | 203 | 82.2 | 44 | 17.8 | 247 | |
| BMI | <19 | 2 | 100.0 | 0 | 0.0 | 2 | 0.039 |
| 19–24 | 62 | 66.7 | 31 | 33.3 | 93 | |
| 25–30 | 149 | 78.4 | 41 | 21.6 | 190 | |
| >30 | 63 | 84.0 | 12 | 16.0 | 75 | |
| Comorbidity | No | 152 | 77.9 | 43 | 22.1 | 195 | 0.532 |
| Yes | 124 | 75.2 | 41 | 24.8 | 165 | |
| Symptom | No | 13 | 86.7 | 2 | 13.3 | 15 | 0.350 |
| Yes | 263 | 76.2 | 82 | 23.8 | 345 | |
| History of hospitalization within the last year | No | 236 | 78.1 | 66 | 21.9 | 302 | 0.130 |
| Yes | 40 | 69.0 | 18 | 31.0 | 58 | |
| History of biliary disease | No | 215 | 81.4 | 49 | 18.6 | 264 | 0.002 |
| Yes | 61 | 63.5 | 35 | 36.5 | 96 | |
| Underwent biliary surgery before cholecystectomy | No | 273 | 77.8 | 78 | 22.2 | 351 | 0.002 |
| Yes | 3 | 33.3 | 6 | 66.7 | 9 | |
| Biliary instrumentation | No | 275 | 77.2 | 81 | 22.8 | 356 | 0.014 |
| Yes | 1 | 25.0 | 3 | 75.0 | 4 | |
| History of antibiotic use in the last 3 months | No | 212 | 75.4 | 69 | 24.6 | 281 | 0.301 |
| Yes | 64 | 81.0 | 15 | 19.0 | 79 | |
| ASA score | 1 | 75 | 77.3 | 22 | 22.7 | 97 | 0.488 |
| 2 | 160 | 78.0 | 45 | 22.0 | 205 | |
| 3 | 39 | 72.2 | 15 | 27.8 | 54 | |
| 4 | 2 | 50.0 | 2 | 50.0 | 4 | |
| Operation simultaneously with cholecystectomy | No | 258 | 77.7 | 74 | 22.3 | 332 | 0.001 |
| Yes | 18 | 64.3 | 10 | 35.7 | 28 | |
| Presence of complication | No | 270 | 78.3 | 75 | 21.7 | 345 | 0.001 |
| Yes | 6 | 40.0 | 9 | 60.0 | 15 | |
| The patients with risk factors* | No | 135 | 81.3 | 31 | 18.7 | 166 | 0.050 |
| Yes | 141 | 72.7 | 53 | 27.3 | 194 | |

*Risk factors: DM, malignancy, immunosuppression, biliary disease, biliary surgery, biliary instrumentation antibiotic use in the last three month, hospitalization the last year*
and other microorganisms \((p = 0.283)\) did not differ according to the risk of growth in culture. Ampicillin resistance in \textit{Enterococci} strains and methicillin resistance in \textit{Staphylococci} strains were determined in cultures of patients with risk. \textit{P. aeruginosa} grew only in cultures of patients with risk (Table 5). Antimicrobial resistance results of Gram-positive and -negative bacteria are presented in Tables 6 and 7.

A total of 20 patients had positive \textit{Staphylococcus} culture, 16 (80\%) of them had different risk factors. The most common risk factors in these patients were biliary disease \((n = 14, 87.5\%)\), DM \((n = 5, 31.2\%)\), history of hospitalization during the previous year \((n = 3, 18.7\%)\), and history of antibiotic use in the last 3 months \((n = 2, 12.5\%)\). Polymicrobial microorganisms were isolated from 22 patients (6.1\%).

Eighty-one patients (22.5\%) underwent surgery while receiving treatment for cholecystitis, and the remaining 279 patients (77.5\%) received prophylactic antibiotics before surgery. Cefazolin was used in 254 patients (91\%) and ceftriaxone in 25 patients (9\%) for surgical prophylaxis. Prophylactic antibiotics were administered to 181 (64.8\%) patients 30 min before the incision. Ninety-one patients (32.6\%) received antibiotics 1 h before the incision and seven (2.5\%) patients at the time of the incision.

### Table 3 Laboratory parameters affecting culture positivity

|                      | Culture negative |          | Culture positive |          |
|----------------------|------------------|----------|------------------|----------|
|                      | \(n\)            | Mean     | SD               | Median   | \(n\)     | Mean     | SD               | Median   | \(p\)     |
| WBC                  | 276              | 8726.9   | 3744.6           | 7820.0   | 84        | 10325.8  | 4853.4           | 8880.0   | 0.017     |
| PNL (%)              | 271              | 61.7     | 11.3             | 59.8     | 83        | 70.7     | 12.6             | 69.8     | 0.001     |
| Hemoglobin           | 276              | 13.1     | 1.7              | 13.2     | 84        | 13.2     | 1.6              | 13.2     | 0.685     |
| PLT                  | 276              | 271.2    | 68.5             | 263.0    | 84        | 254.2    | 88.9             | 250.5    | 0.066     |
| CRP                  | 156              | 3.7      | 6.7              | 0.8      | 67        | 6.3      | 8.8              | 1.3      | 0.019     |
| Creatinine           | 271              | 0.8      | 0.6              | 0.7      | 82        | 0.8      | 0.3              | 0.8      | 0.008     |
| AST                  | 272              | 25.1     | 23.9             | 19.0     | 84        | 32.2     | 35.3             | 20.0     | 0.224     |
| ALT                  | 272              | 31.1     | 41.2             | 20.5     | 83        | 45.3     | 70.6             | 22.0     | 0.486     |
| ALP                  | 255              | 84.6     | 37.6             | 76.0     | 79        | 102.1    | 73.1             | 85.0     | 0.010     |
| GGT                  | 258              | 56.5     | 109.2            | 26.0     | 79        | 72.2     | 124.8            | 29.0     | 0.505     |
| T. bilirubin         | 262              | 0.6      | 0.4              | 0.5      | 82        | 0.9      | 1.0              | 0.7      | 0.003     |
| Glucose              | 270              | 107.4    | 33.9             | 100.0    | 84        | 116.8    | 35.0             | 111.0    | 0.002     |

\(WBC\) white blood cell, \(PNL\) polymorph nuclear leukocyte, \(PLT\) platelet, \(AST\) aspartate aminotransferase, \(ALT\) alanine aminotransferase, \(ALP\) alkaline phosphatase, \(GGT\) gamma glutamyl transferase

### Table 4 Multivariate analysis of factors associated with culture positivity

|                      | \(B\)     | S.E    | \(p\)     | OR   | Lower | Upper |
|----------------------|-----------|--------|-----------|------|-------|-------|
| History of biliary disease (Yes/No) | 1.058     | 0.372  | \textbf{0.004} | 2.880 | 1.390 | 5.965 |
| Conversion from laparoscopy to open surgery (Yes/No) | 2.369     | 1.122  | \textbf{0.035} | 10.691 | 1.186 | 96.352 |
| Operation simultaneously with cholecystectomy (Yes/No) | 1.704     | 0.699  | \textbf{0.015} | 5.497 | 1.397 | 21.626 |
| \(PNL\) (%)          | 0.060     | 0.017  | \textbf{0.000} | 1.061 | 1.027 | 1.096 |
| Constant              | -7.140    | 1.590  | 0.000     | 0.001 |       |       |

\(PNL\) polymorphonuclear leukocytes

(p = 0.406), and other microorganisms \((p = 0.283)\) did not differ according to the risk of growth in culture. Ampicillin resistance in \textit{Enterococci} strains and methicillin resistance in \textit{Staphylococci} strains were determined in cultures of patients with risk. \textit{P. aeruginosa} grew only in cultures of patients with risk (Table 5). Antimicrobial resistance results of Gram-positive and -negative bacteria are presented in Tables 6 and 7.

A total of 20 patients had positive \textit{Staphylococcus} culture, 16 (80\%) of them had different risk factors. The most common risk factors in these patients were biliary disease \((n = 14, 87.5\%)\), DM \((n = 5, 31.2\%)\), history of hospitalization during the previous year \((n = 3, 18.7\%)\), and history of antibiotic use in the last 3 months \((n = 2, 12.5\%)\). Polymicrobial microorganisms were isolated from 22 patients (6.1\%).

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### Discussion

Cholecystectomy is one of the most common abdominal surgical procedures and often performed due to acute cholecystitis and other complications of gallstones (Jaafar et al. 2017; Potts 1990). Sufficient data concerning the bacteriology of bile in patients undergoing cholecystectomy for acute cholecystitis do not exist in the literature (Coccolini et al. 2015). Previous studies reported that the rate of bile culture positivity ranged from 9 to 42\% (Yun and Seo 2018; Galili et al. 2008; Mahafzah and Daradkeh 2009). In our study, the microorganisms were determined in 23.3\% of the patients who underwent cholecystectomy, and our results were thought to be similar to previous studies.

\textit{E. coli}, \textit{Enterococcus} spp., \textit{Klebsiella} spp., and \textit{Enterobacter} spp. are the most commonly identified microorganisms in bile culture (Coccolini et al. 2015; Yun and Seo 2018). These bacteria are typically found in the intestinal flora and migrate...
ascending through the duodenum to colonize the gallbladder. E. coli, Enterobacter spp., and Klebsiella spp. were the most frequently isolated bacteria in our non-risk patients.

| Enterococci spp. | Risk factors                                      | n | % | n | % | p |
|------------------|--------------------------------------------------|---|---|---|---|---|
| Ampicillin resistance | No | 0 | 0.0 | 9 | 100.0 | 0.018 |
| Ampicillin sensitive | No | 8 | 53.3 | 7 | 46.7 |   |
| Methicillin resistance | No | 0 | 0 | 10 | 100.0 | 0.007 |
| Methicillin sensitive | No | 3 | 30.0 | 7 | 70.0 |   |
| ESBL positive | No | 3 | 75.0 | 1 | 25.0 | 0.606 |
| ESBL negative | No | 7 | 53.8 | 6 | 46.2 |   |
| ESBL positive | Yes | 2 | 66.7 | 1 | 25.0 | 0.381 |
| ESBL negative | Yes | 1 | 33.3 | 3 | 75.0 |   |
| Pseudomonas spp. | Culture positivity | No | 164 | 46.2 | 191 | 53.8 | 0.039 |
| | Yes | 0 | 0.0 | 5 | 100.0 |   |
| Culture positivity | No | 135 | 48.9 | 141 | 51.1 | 0.050 |
| | Yes | 31 | 36.9 | 53 | 63.1 |   |
| Two bacteria | No | 158 | 46.7 | 180 | 53.3 | 0.344 |
| | Yes | 8 | 36.4 | 14 | 63.6 |   |

* Risk: DM, malignity, autoimmune disorder, biliary disease, biliary surgery, biliary instrumentation, antibiotic use in last month. Hospitalization the last year

** Others: Acinetobacter baumannii, Corynebacterium spp., Haemophilus spp., Rothia dentocariosa and Candida spp. in one patient for each

| Enterobacterales* | Non-fermentative Gram-negative bacilli** |
|-------------------|----------------------------------------|
| n | 39 | n | 6 |
| n | % | n | % |
| AMC | 9/26 | 34.6 | – | – |
| Cefuroxime | 12/31 | 38.7 | – | – |
| Ceftriaxone | 7/32 | 21.8 | – | – |
| Ceftazidime | 7/32 | 21.8 | 2/4 | 50 |
| Cefepime | 6/30 | 20 | 3/5 | 60 |
| PIPTAZ | 8/31 | 25.8 | 1/4 | 25 |
| Gentamycin | 5/23 | 21.7 | 0/3 | 0 |
| Amikacin | 0/27 | 0 | 1/5 | 20 |
| Ciprofloxacin | 4/30 | 13.3 | 2/4 | 50 |
| Ertapenem | 2/29 | 6.8 | – | – |
| IMP/MEM | 0/28 | 0 | 2/6 | 33.3 |
| SXT | 4/30 | 13.3 | – | – |
| Tigecycline | 4/20 | 20 | – | – |

AMC amoxicillin-clavulanic acid, PIPTAZ piperacillin-tazobactam, IMP/MEM imipenem/meropenem, SXT trimethoprim/sulfamethoxazole

* Enterobacterales: E. coli (n = 19), Klebsiella spp. (n = 10), Enterobacter spp. (n = 7), and Citrobacter spp. (n = 3); non-fermentative Gram-negative bacilli: P. aeruginosa (n = 5) and A. baumannii (n = 1)

** High level of aminoglycoside resistance (HLAR) was investigated in Enterococcus spp.

Other Gram-positive bacteria: Staphylococcus spp. (n = 22) and Streptococcus spp. (n = 8)

Staphylococci spp. were the most common bacteria among at-risk patients in our study. We determined that most of the patients with isolated staphylococcus had a history of biliary disease or a history of hospitalization during the previous year or antibiotic use over the last 3 months.

Table 5: Cultures and antimicrobial test results of isolates according to presence of risk factors.

Table 6: Antimicrobial resistance results of Gram-negative bacilli.

Table 7: Antimicrobial resistance results of Gram-positive bacteria.
Staphylococcus spp. have grown in the bile culture in some studies, including the present study, even though it is not part of the enteric flora (Coccolini et al. 2015; Darkahi et al. 2014). These bacteria are thought to possibly be of enteric origin due to changes in intestinal microbial flora and are less likely to reach the gallbladder via hematogenous route (Darkahi et al. 2014).

Gallbladder disease is known to occur, especially in middle-aged women (Katyal et al. 2017). Therefore, multiple studies on gallbladder cultures have shown a higher percentage of women patients in their cohorts (Darkahi et al. 2014; Katyal et al. 2017). The majority of the patients in our study were women, similar to the study conducted by Galili et al. (Galili et al. 2008). In our study, culture positivity was found to be higher in men in univariate analysis even though most of the study group consisted of women. When multivariate analysis was performed, it was determined that culture positivity was not statistically significant in males.

The study of Mahafzah et al. determined that advanced age and pre-operative endoscopic retrograde cholangiopancreatography (ERCP) were significant risk factors for a positive bile culture (Mahafzah and Daradkeh 2009). Galili et al. demonstrated that advanced age, increased leukocyte count, high serum bilirubin, and high ALP levels accompanied bactobilia. The author in this study reported that the increase in laboratory values in patients with growth on the bile culture may be a sign of advanced gallbladder infection (Galili et al. 2008). In our study, advanced age, history of biliary disease, undergoing biliary surgery before cholecystectomy, presence of biliary instrumentation, and an increase in leukocyte count, PNL ratio, CRP, creatinine, ALP, glucose levels, and total bilirubin values were found to be significant predictors of positive bile culture in univariate analysis. History of biliary disease, operation performed concurrently with a cholecystectomy, and a high PNL rate in laboratory results were found to be statistically significant in the multivariate analysis. Although the conversion from a laparoscopic operation to an open operation was significant in the multivariate analysis, it was not deemed significant due to the small sample size.

Capoor et al. demonstrated that 4.8% of the patients had polymicrobial infection in the gallbladder (Capoor et al. 2008). Yun et al. stated that two bacteria were identified together in the bile culture of 51.9% of the patients. In their study, they demonstrated that low BMI, previous ERCP, and presence of operative complications were significantly associated with multiple growths in bile culture (Yun and Seo 2018). In our study, polymicrobial growth was found in 6.1% of the patients. Although the results were not statistically significant, polymicrobial growth was higher in the risk group patients. Culture positivity and presence of resistant bacteria were found to be statistically significantly higher in the risk group patients.

Risk factors for multi-drug resistant bacteria were found to be DM, immunosuppression, and previous antibiotic therapy and hospitalization history. These risk factors should be evaluated while organizing the empirical treatment (Iribar et al. 2017). In a multi-center prospective study of patients with acute cholecystitis, 267 bacteria were isolated in the gallbladder culture of 567 patients, and 21 (7.8%) of these bacteria were resistant. Carbapenem resistance was detected in one A. baumannii strain and one K. pneumoniae strain. ESBL positivity was detected in 12 E. coli strains and four K. pneumoniae strains, with glycopenpeptide resistance in enterococci isolated in one strain and methicillin resistance isolated in staphylococci in two strains (Coccolini et al. 2015). In our study, MDR Gram-positive bacteria were isolated in 19 strains. Carbapenem resistance was determined in two non-fermentative Gram-negative bacilli. ESBL-E were detected in 10 Enterobacterales strains. Although ESBL-positive strains are frequently isolated in patients with risk, they have also been detected in without-risk patients. Ciprofloxacin resistance was determined to be 21% in Enterococci spp. and 13.3% in Enterobacterales strains, when risk factors were not taken into account. According to these results, it was considered that antimicrobial resistance was a concern, and caution should be necessary when applying empirical therapy.

Administration of antimicrobial prophylaxis is recommended 60 min before surgical incision in patients undergoing either high risk laparoscopic or open cholecystectomy. Although antimicrobial prophylaxis is not recommended in low-risk patients, it is reported that it may be rational to give antimicrobial prophylaxis to all patients undergoing laparoscopic cholecystectomy since high risk cannot be determined before surgical intervention (https://www.ashp.org/surgical-guidelines). Infection rates after laparoscopic cholecystectomy were reported to be 0–7% and 0–4% in patients with and without antimicrobial prophylaxis, respectively (https://www.ashp.org/surgical-guidelines). Our study showed that all patients who underwent biliary surgery were either under treatment or received applied surgical prophylaxis. Considering the increasing resistance rates, the need for surgical prophylaxis should be re-evaluated in patients without infection risk.

One limitation of our study is the small number of cases. However, we believe that the results are important because it is a national multicenter study. Another limitation of our study was the inability to perform metagenomic analysis. Microbial culture has limitations and may miss several relevant microbes. However, it is possible to determine the microorganisms more comprehensively with metagenomic analysis (Dyrhovden et al. 2020). Due to the high cost of metagenomic analysis and the lack of financial support, only preoperative
specimen cultures and growth agent antibiotic susceptibility patterns could be investigated. Another limitation of this study was that anaerobic culture was not performed to all the samples.

A history of biliary disease, operation performed concurrently with a cholecystectomy, and a high PNL rate in total leukocyte count increased the growth rate in bile culture. Since biliary tract infections can be fatal, it is vital to follow up the development of antimicrobial resistance. Evaluating individual risks in patients with biliary tract infections, while planning empirical antibiotic therapy, will help ensure rational antibiotic use.

Author contribution All the authors (D. Ozturk-Engin, C. Agalar, Y. Cag, F. Kesmez-Can, I. I. Balkan, O. Karabay, S. Senbayar, B. Meral-Cetinkaya, M.T. Aydn, K. Tomas, E. Disci, A. Surmelioğlu, O. Alimoğlu, O. Ekinci, E. Akin, M. Koroğlu, M. Velidedecoglu, H. Ankarali, E. Kocoglu, E. Ozmen, M. Javadov, B. Papilla-Kundaktepe, N. Oguuzoglu, R. Donmez, E. Mega, S. Aksaray, F. Agalar) have made significant contributions to the concept or design of the study. Material preparation, data collection, and analysis were performed by these authors. They drafted and critically reviewed the study. They have contributed to and are responsible for all aspects of his work. The final version of the article has been read and approved by all authors. All named authors have seen and reviewed the submitted version of the paper and agreed to all included data.

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Data availability The datasets for this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Fatih Sultan Mehmet Training and Research Hospital in Istanbul, Turkey. Our study was designed prospectively and approval was obtained from the local ethics committee before the study started. The ethics committee document obtained from the ethics committee is attached. Ethics committee document is written in Turkish. However, if requested, we can send it in English. In our study, samples taken preoperatively were examined.

Conflict of interest The authors declare no competing interests.

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