의학석사 학위논문

급성 담낭염 환자에서 담즙 배양의 미생물학 및 항생제 감수성에 따른 경험적 항생제 선택에 대한 연구

Selection of empirical antibiotics in acute cholecystitis based on bile microbiology and antibiotic susceptibility

2023년 2월

서울대학교 대학원
의학과 외과학 전공
이 정 민

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지도교수 장 진 영

이 논문을 의학석사 학위논문으로 제출함

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이 정 민

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Abstract

Selection of empirical antibiotics in acute cholecystitis based on bile microbiology and antibiotic susceptibility

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Background: Bacterial infection is common in acute cholecystitis (AC). To identify appropriate empirical antibiotics, we investigated AC-associated microorganisms and their susceptibilities to antibiotics. We also compared preoperative clinical findings of patients grouped according to specific microorganisms.

Methods: Patients who underwent laparoscopic cholecystectomy for AC between 2018 and 2019 were enrolled. Bile cultures and antibiotic susceptibility tests were performed, and clinical findings of patients were noted.

Results: A total of 282 patients were enrolled (147 culture-positive and 135 culture-negative). The most frequent microorganisms were *Escherichia* (n=53, 32.7%), *Enterococcus* (n=37, 22.8%), *Klebsiella* (n=28, 17.3%), and *Enterobacter* (n=18, 11.1%). For Gram-negative microorganisms, second-generation cephalosporin (cefotetan: 96.2%) was more effective than third-generation cephalosporin (cefotaxime: 69.8%). Vancomycin and teicoplanin (83.8%) were the most effective antibiotics for *Enterococcus*. Patients with *Enterococcus* had higher rates of CBD stones (51.4%, *p*=0.001) and biliary drainage (81.1%, *p*=0.002), as well as higher levels of liver enzymes (*p*=0.001), than patients with other microorganisms. Patients with ESBL-producing bacteria had higher rates of CBD stones (36.0% vs. 6.8%, *p*=0.001) and biliary drainage (64.0% vs. 32.4%, *p*=0.005) than those without.

Conclusion: Preoperative clinical findings of AC are related to the genus of microorganisms in bile samples. Periodic antibiotic susceptibility tests should be conducted to select appropriate empirical antibiotics.

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Keywords : Cholecystitis; Microbiology; Antibiotics; Microbial sensitivity test

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I. Introduction

Acute cholecystitis (AC) is usually caused by obstruction of the cystic duct by gallbladder (GB) stones or biliary sludge.[1] Secondary bacterial infection with enteric organisms occurs in 50% to 85% of AC cases.[2] The routes of infection include ascending infection from the duodenum, descending infection from the liver, hematogenous infection from the hepatic artery or portal vein, and infection through lymphatics.[3] Infection of the biliary tract can lead to septic shock and death[4-5]; therefore, infection control through the use of appropriate antibiotics plays a pivotal role in the treatment of AC.

The microorganisms identified in AC cases differ depending on the time, geographical regions, institutions, and individuals.[6-7] Therefore, periodic research into treatments that are suitable for the local community is necessary. The past few years have seen an increase in the prevalence of antimicrobial-resistant strains, such as Gram-negative bacilli, which produce extended-spectrum beta-lactamase (ESBL) or carbapanemase.[8-9] Infection caused by ESBL-producing bacteria is associated with adverse patient outcome and increased treatment costs.[10-11] Consequently, the emergence of such strains has made the selection of empirical antibiotics more difficult and important.

Early empirical antibiotic-based eradication of causative microorganisms is essential for AC patients and cannot be delayed while clinicians wait for the results of culture tests. Several studies have attempted to recommend appropriate empirical antibiotics for AC by identifying commonly isolated microorganisms and their antibiotic susceptibilities.[12-15] According to Tokyo Guideline 2018 (TG18), *Escherichia* and *Klebsiella* are the most common bacteria isolated from patients with biliary tract infections.[16] Depending on the severity of AC, TG18 recommends empirical therapy with penicillin, cephalosporin, fluoroquinolone, monobactam, and carbapenem. Other studies have also recommended the use of empirical antibiotic therapy according to the severity of disease.[17-18] Therefore, our current study was designed to investigate the microorganisms commonly associated with AC and their antibiotic susceptibilities. We also compared the preoperative clinical findings of patients grouped according to specific microorganisms, with the aim of proposing appropriate empirical antibiotics.

II. Methods

Patients

A total of 845 patients underwent laparoscopic cholecystectomy at Seoul National University Boramae Medical Center between January 2018 and December 2019. Among 845 patients, 53 patients diagnosed as having GB polyps (or cancer) or received combined surgery, and 103 patients for whom bile cultures were missing were excluded from study. In addition, 407 patients who did not meet the criteria for AC but had symptoms or radiographic findings of cholecystitis were diagnosed as having chronic cholecystitis. Consequently, 282 patients diagnosed as having AC were enrolled in the study (Figure 1). The TG18 criteria for AC diagnosis and severity assessment were used.[19] All patients included in the study had bile culture results and were divided into culture-positive and culture-negative groups for comparison. we defined polymicrobial infection as two or more bacterial species present in a culture test. The study was approved by the institutional review board of Seoul National University Boramae Medical Center (IRB No. 30-2021-15), with a waiver for informed consent.

Data collection

The baseline characteristics of the patients enrolled in the study, including age, sex, underlying disease, previous operation history, and American Society of Anesthesiologists (ASA) status, were recorded. Preoperative clinical findings, including initial symptoms, radiologic findings, laboratory results, and severity grade (by TG18), were noted. Patients with a body temperature of 38°C or higher were defined as having a fever. The presence of GB stones, common bile duct (CBD) stones and cholangiohepatitis was evaluated by computed tomography or ultrasonography. Percutaneous transhepatic gallbladder drainage (PTGBD) was performed in patients who had grade 3 cholecystitis, or CBD stone requiring endoscopic retrograde cholangiopancreatography for removal, or severe comorbidity that needs preoperative evaluation.

The number of patients who received biliary drainage was also recorded. Intraoperative findings, including operation time, biliary tract injury, bile leakage, and intra-abdominal drainage insertion, were also recorded. In addition, postoperative outcomes, including fever, use of additional antibiotics, laboratory results, duration of hospital stay, and complications, were investigated.

Bile cultures and antibiotic susceptibility testing

Bile swabs were performed in an aseptic manner immediately after retrieval of the GB specimen. In patients who received PTGBD before surgery, culture was performed using the bile collected during the procedure. The bile samples were inoculated on blood agar plates followed by McConkey agar plates. The plates were then incubated at 35°C and 5% carbon dioxide in an incubator (Thermo Scientific, Inc., Waltham, MA, USA) for 1 day. Gram staining was performed in the presence of colonies on inoculated culture medium. Subsequently, the specimens were inoculated on Mueller–Hinton agar and tested for susceptibility to antibiotics using the disk diffusion method.

Analysis of clinical findings in patients infected with specific microorganisms

Clinical findings were compared between patients with the four most commonly isolated bacteria. The preoperative clinical findings included initial symptoms, radiologic findings, laboratory results, severity grade (by TG18), and implementation of biliary drainage. We also compared the clinical findings of patients with and without ESBL-producing microorganisms, which were defined as those showing resistance to third-generation cephalosporins and monobactam.

Statistical analysis

Continuous variables were calculated as the mean and standard deviation, and were analyzed using the Student's t-test or Mann–Whitney U-test, as appropriate. Categorical variables were compared using the Chi-squared test or Fisher's exact test. Statistical significance was defined as $p<0.05$. All statistical calculations were performed using SPSS software (SPSS Statistics 25.0, SPSS, Inc., Chicago, IL, USA).

III. Results

Demographic and preoperative clinical findings

The baseline characteristics and preoperative clinical findings of the patients enrolled in the study are shown in Table 1. Among the 282 patients, 147 (52.1%) were culture-positive. On average, the culture-positive patients were older than the culture-negative patients (68.0 vs. 58.5 years, p=0.001). In addition, more patients had hypertension (53.1% vs. 36.3%, p=0.005) and a history of previous operations (32.0% vs. 20.0%, p=0.022) in the culture-positive group than in the culture-negative group. The most common type of previous operation was gynecological surgery (31.1%) such as cesarean section or hysterectomy, followed by lower gastrointestinal surgery (29.7%), upper gastrointestinal surgery (23.0%), and urologic surgery (10.8%). The ASA status also differed significantly between the culture-positive and culture-negative groups (class I: 12.9% vs. 37.8%; class II: 66.7% vs. 43.7%, respectively; p=0.001).

Compared with the culture-negative patients, the culture-positive patients demonstrated fever (37.4% vs. 25.9%, p=0.039) and displayed radiographic findings of CBD stones (25.9% vs. 8.9%, p=0.001) more frequently. The levels of leukocytosis (white blood cell count: 14200.2 vs. 11648.5 /μL, p=0.001), C-reactive protein (14.6 vs. 7.9 mg/L, p=0.001), and alkaline phosphatase (140.6 vs. 108.9 IU/L, p=0.012) were higher in the culture-positive group than in the culture-negative group. The prevalence of TG18-defined grade I AC was higher in the culture-negative group than in the culture-positive group (49.7% vs. 68.9%), whereas those of grades II and III AC were higher in the culture-positive group (grade II: 45.6% vs. 28.1%; grade III: 4.8% vs. 3.0%, p=0.005). In addition, the numbers of patients receiving PTGBD (35.4% vs. 23.0%) or endoscopic retrograde biliary drainage (ERBD; 27.2% vs. 8.9%) were significantly higher in the culture-positive group than in the culture-negative group (p=0.002).

Perioperative short-term outcomes

The intraoperative findings and the postoperative outcomes are shown in Table 2. The culture-positive patients had longer mean operation times (51.1 vs. 43.7 min, p=0.007) and were more likely to suffer biliary duct injury (3.4% vs. 0.0%, p=0.031) and bile leakage (21.8% vs. 8.1%, p=0.001) than the culture-negative patients. In addition, an intra-abdominal drainage tube was more common in the culture-positive group (63.3% vs. 37.8%, p=0.001).

After surgery, the proportion of patients who received antibiotics was larger in the culture-positive group than in the culture-negative group (76.2% vs. 63.0%, p=0.001). In addition, culture-positive patients demonstrated higher mean levels of C-reactive protein than culture-negative patients (11.5 vs. 7.1 mg/L, p=0.002). Culture-positive patients tended to stay in hospital longer than culture-negative patients (4.8 vs. 3.7 days), but the difference was not statistically significant (p=0.214). The incidence of complications with Clavien Dindo grade 3a or higher was comparable between the culture-positive and culture-negative groups (11.6% vs. 14.1%, respectively; p=0.528).

Identified microorganisms and antibiotic susceptibility

Among the 282 patients enrolled in the study, 162 microorganisms were identified from 147 culture-positive patients (52.1%). Thirteen of 147 culture-positive patients showed polymicrobial infection (Supplementary table 1). The four most frequently identified microorganisms were *Escherichia* (n=53, 32.7%), *Enterococcus* (n=37, 22.8%), *Klebsiella* (n=28, 17.3%), and *Enterobacter* (n=18, 11.1%) (Figure 2). Other microorganisms, such as *Citrobacter*, *Streptococcus*, and *Staphlyococcus*, were also isolated from 20 patients. In addition, *Candida* was isolated from 6 patients.

Table 3 shows the proportions of the four most commonly isolated microorganisms that were susceptible to various antibiotics. For cephalosporin antibiotics, cefotetan (second generation) was more effective than cefotaxime (third generation) in eradicating *Escherichia* (96.2% vs. 69.8%) and *Klebsiella* (100% vs. 96.4%). On the other hand, ceftazidime was the most effective cephalosporin antibiotic against *Enterobacter* (77.8%). Ciprofloxacin was effective against *Klebsiella* (89.3%) and *Enterobacter* (94.4%), but only eradicated 60.4% of *Escherichia* cases. Amikacin (*Escherichia*, 96.2%; *Klebsiella* and *Enterobacter*, 100%) and imipenem (*Escherichia*, *Klebsiella*, and *Enterobacter*, 100%) were the most effective antibiotics against Gram-negative microorganisms. Among the four most commonly isolated microorganisms, *Enterococcus* was the only Gram-positive bacteria identified. Vancomycin and teicoplanin were effective in 31 of 37 patients (83.8%) infected with *Enterococcus*, but the remaining patients displayed resistance to these antibiotics.

Clinical findings in patients infected with specific microorganisms

Table 4 shows the clinical features of patients grouped according to infection with specific microorganisms. At the time of admission, fever was more frequent in patients with *Enterococcus* than in other patients with Gram-negative microorganisms (*Escherichia*, 35.8%; *Klebsiella*, 50.0%; *Enterobacter*, 22.2%; *Enterococcus*, 54.1%; p=0.084). Based on radiologic findings, GB stones were observed more frequently in patients with *Enterobacter* (94.4%) or *Enterococcus* (89.2%) than in those with *Escherichia* (71.7%) or *Klebsiella* (75.0%) (p=0.017). Among the groups examined, patients with *Enterococcus* were the most prone to CBD stones with dilatation (*Escherichia*, 11.3%; *Klebsiella*, 7.1%; *Enterobacter*, 33.3%; *Enterococcus*, 51.4%; p=0.001) and cholangiohepatitis (*Escherichia*, 15.1%; *Klebsiella*, 7.1%; *Enterobacter*, 11.1%; *Enterococcus*, 43.2%; p=0.001). Liver function tests revealed that the serum levels of total bilirubin, aspartate aminotransferase, and alanine aminotransferase were higher in patients with *Enterococcus* than in those with other microorganisms. Biliary drainage was performed most frequently in patients with *Enterococcus*, followed by those with *Enterobacter*, *Escherichia*, and then *Klebsiella*.

Clinical findings in patients infected with and without ESBL-producing Gran-negative bacteria

Among the 99 patients who were only infected by gram-negative microorganisms, ESBL-producing bacteria were identified in 25 (25.3%) patients. Table 5 shows the clinical features of patients infected with or without ESBL-producing microorganisms. Patients with

ESBL-producing bacteria were more likely to have a fever than those without (44.0% vs. 29.7%, p=0.191) and were more prone to CBD stones with dilatation (36.0 vs. 6.8%, p=0.001) and cholangiohepatitis (28.0% vs. 4.1%, p=0.001). Preoperative biliary drainage was performed more commonly in patients with ESBL-producing bacteria than in those without (64.0% vs. 32.4%, p=0.005). In addition, patients with ESBL-producing bacteria had higher serum levels of aspartate aminotransferase (193.3 vs. 68.9 IU/L, p=0.015) and alanine aminotransferase (138.8 vs. 38.6 IU/L, p=0.015) before surgery than those without.

Selection of empirical antibiotics according to the type of preoperative biliary drainage

As clinical features were compared according to the species of microorganisms, CBD stone and preoperative biliary drainage were the most important factors. Therefore, we investigated the distribution of microorganisms according to the type of preoperative biliary drainage (Table 6). Gram-negative microorganisms without ESBL were predominated in patients who received upfront surgery or PTGBD (p=0.001). On the other hand, there were more patients with gram-positive or ESBL-producing microorganisms in PTBD and ERBD group. Based on these differences, we proposed a flow chart to select empirical antibiotics according to the type of preoperative biliary drainage (Figure 3). For upfront surgery and PTGBD group, cefotetan (second-generation cephalosporin) was recommended, which were highly sensitive for gram-negative microorganism in our study. For PTBD and ERBD group, cefotetan plus ampicillin (aminopenicillin) was recommended, which could cover both gram-negative and positive microorganisms. If these antibiotic treatments are ineffective, change to antibiotics such as amikacin, imipenem, vancomycin and teicoplanin should be considered.

IV. Discussion

Appropriate empirical antibiotic therapy for AC patients is important because secondary bacterial infection leads to poor surgical outcomes. Furthermore, inappropriate initial antimicrobial therapy is associated with increased mortality in patients with biliary tract infection. In 2008, Galili et al.[20] reported that culture-positive AC patients had higher postoperative complication rates than culture-negative AC patients (24.7% vs. 10.7%). Correspondingly, in our study, intraoperative biliary tract injury and bile leakage were more common in culture-positive patients than in culture-negative patients. The duration of postoperative fever and hospital stay also tended to be longer in the culture-positive group, although the difference was not statistically significant. Although there were no mortalities in our study, Kang et al.[21] performed a multivariate analysis of patients with biliary tract infection and reported that the use of inappropriate initial antibiotics was associated with increased 30 day mortality.

TG18 recommends the use of third-generation cephalosporin for grades II and III AC, rather than second-generation cephalosporin.[16] The majority of AC patients (63.0%) seen at our institution are administered a combination of third-generation cephalosporin and metronidazole as empirical antibiotics. However, in our current study, second-generation cephalosporin (cefotetan: 96.2%) was more effective against *Escherichia* than third-generation cephalosporin (cefotaxime: 69.8%). In addition, second-generation cephalosporin was more effective against other Gram-negative microorganisms (*Klebsiella* and *Enterobacter*) than third-generation cephalosporin. We think this finding can be explained by an increased prevalence of resistant strains due to the continuous use of third-generation cephalosporins in our community. The specific microorganisms commonly identified in AC could differ across different time-periods and geographical regions[6-7]; therefore, it is important to check the antibiotic susceptibility of the local community periodically.

In our current study, the rates of ERBD and PTGBD were higher in the culture-positive group than in the culture-negative group because the rate of occurrence of CBD stones on initial radiologic images was higher in the former than in the latter. In addition, the culture-positive group had a higher TG18 grade of AC with fever and a higher level of leukocytosis than the culture-negative group. Similarly, in 2018, Yun et al.[22] reported that the rates of ERBD and PTGBD were significantly higher in culture-positive AC patients than in culture-negative patients. Therefore, we propose that patients who have fever, leukocytosis, a high TG18 grade, and preoperative biliary drainage should be treated with more aggressive antibiotic therapies.

Enterococcus, which was the only genus of Gram-positive bacteria identified among the four most commonly isolated microorganisms, was identified in 37 patients in our study. Eradication of *Enterococcus* requires different antibiotics to those used to eradicate Gram-negative bacteria. In our current study, GB stones were more common in patients with *Enterococcus* than in those with other microorganisms. These findings differ from those of previous studies, in which *Escherichia* was the most frequently identified microorganism in patients with cholelithiasis.[23-24] These contradictory findings might be related to differences in geographical regions and time-points, and/or to the use of small sample sizes. Here, the rates of CBD stones with dilatation and preoperative biliary drainage were higher in patients with *Enterococcus* than in those with other microorganisms, as were the levels of liver enzymes. Our findings suggest that patients presenting with GB stones, CBD stones, and/or increased serum levels of liver enzymes would benefit from treatment with vancomycin or teicoplanin, both of which were effective against *Enterococcus* (83.8%) in our current study.

Selection of appropriate empirical antibiotics requires careful consideration of resistant bacteria. In particular, ESBL-producing *Enterobacteriaceae* is common in AC. In a prospective cohort study performed in 2015, Coccolini et al.[25] reported that 16 (16.7%) of the 96 *Escherichia coli* species identified in AC patients were

ESBL-producing bacteria. Similarly, in our current study, antibiotic susceptibility testing revealed that, among the 112 Gram-negative microorganisms identified, 28 (25.0%) were ESBL-producing bacteria. The rates of fever and CBD stones with dilatation were higher in patents with ESBL-producing bacteria than in those without them, as were the serum levels of liver enzymes. For these patients, TG18 recommends the use of antibiotics such as carbapenem, piperacillin/tazobactam, and aminoglycoside. In our study, amikacin (92.9%) and imipenem (100%) were highly effective against ESBL-producing microorganisms. We propose that patients presenting with fever, CBD stones, and increased levels of liver enzymes should be considered for treatment with antibiotics such as carbapenem, piperacillin/tazobactam, and aminoglycoside, depending on the antibiotic susceptibility of the community.

In the flow chart for empirical antibiotic selection proposed in this study, the most important criterion is the presence and type of preoperative biliary drainage. In 2007, Limongelli et al.[26] reported that preoperative biliary drainage predisposed positive intraoperative bile culture, which increased risk of developing infectious complications. Also, in 2014, Sudo et al.[27] reported that preoperative biliary drainage increased not only positive intraoperative bile culture but also antibiotic resistance. They mentioned the need for perioperative antibiotic strategy particular to preoperative biliary drainage procedures. However, there were no previous studies that specifically recommended empirical antibiotics according to the types of preoperative biliary drainage. Therefore, this is the first study to suggest specific empirical antibiotics by examining the distribution of microorganisms according to the type of biliary drainage. While cefotetan was recommended for patients in upfront surgery and PTGBD group, cefotetan plus ampicillin was recommended for patients in PTBD and ERBD group because the proportion of patients with gram-positive microorganisms was significantly higher. If these antibiotics had no therapeutic effect for the patient, amikacin or imipenem were recommended for gram-negative microorganisms and vancomycin or teicoplanin for gram-positive bacteria, depending on the results of the culture test.

The present study has some limitations. First, it was a retrospective study which was conducted in a single institution with a small cohort. Therefore, there could be selection bias. Multinational and multicenter studies should be performed to enable more meaningful comparisons. Second, the efficacy of metronidazole, which is used widely as an empirical antibiotic, was not analyzed. To identify patients who would benefit most from metronidazole therapy, additional studies are required in which patients are grouped into those with aerobes and those with anaerobes. Finally, the results of our study cannot be applied to all geographical regions because they are based on bile cultures and antibiotic susceptibility testing at a specific institution. Antibiotic susceptibility could vary by region and time. Nonetheless, our study may be helpful for local community hospitals that provide primary care for AC.

In conclusion, we found that the clinical characteristics of AC patients at the time of admission are related to the species of microorganism and antibiotic susceptibility. In particular, CBD stones and preoperative biliary drainage are associated with positive culture results and the presence of ESBL-producing bacteria and *Enterococcus*. Finally, we recommended cefotetan for the group that underwent upfront surgery or PTGBD, and cefotetan plus ampicillin for the group that underwent PTBD or ERBD. Periodic antibiotic susceptibility testing should be conducted to select appropriate empirical antibiotics for specific communities.

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Table 1. Baseline characteristics and preoperative findings

Variables	Total (n = 282)	Culture (+) (n = 147)	Culture (−) (n = 135)	P value
Age (years)	63.5 ± 15.7	68.0 ± 12.8	58.5 ± 17.1	0.001
Male sex	175 (62.1%)	93 (63.3%)	82 (60.7%)	0.663
Underlying disease				
Hypertension	127 (45.0%)	78 (53.1%)	49 (36.3%)	0.005
Myocardial infarction	19 (6.7%)	9 (6.1%)	10 (7.4%)	0.667
Previous operation history	74 (26.2%)	47 (32.0%)	27 (20.0%)	0.022
ASA physical status				0.001
I	70 (24.8%)	19 (12.9%)	51 (37.8%)	
II	157 (55.7%)	98 (66.7%)	59 (43.7%)	
III	49 (17.4%)	26 (17.7%)	23 (17.0%)	
Admission				0.216
Emergency room	235 (83.3%)	127 (86.4%)	108 (80.0%)	
Outpatient clinic	29 (10.3%)	14 (9.5%)	15 (11.1%)	
Inpatient consultation	18 (6.4%)	6 (4.1%)	12 (8.9%)	
Symptoms				
Abdominal pain	263 (93.3%)	138 (93.9%)	125 (92.6%)	0.667
Fever (≥38.0°C)	90 (31.9%)	55 (37.4%)	35 (25.9%)	0.039
Jaundice	9 (3.2%)	7 (4.8%)	2 (1.5%)	0.117
Radiologic findings				
Gallbladder stone	225 (79.8%)	119 (81.0%)	106 (78.5%)	0.611
CBD stone & dilatation	38 (13.5%)	38 (25.9%)	12 (8.9%)	0.001
Lab findings				
WBC (/μL)	12978.7 ± 6100.1	14200.2 ± 65345.6	11648.5 ± 5300	0.001
CRP (mg/L)	9.8 ± 10.5	14.6 ± 17.9	7.9 ± 12.1	0.001
Total bilirubin (mg/dL)	2.4 ± 4.7	2.5 ± 2.5	2.2 ± 6.3	0.504
AST (IU/L)	118.0 ± 210.9	133.6 ± 238.3	101.1 ± 175.5	0.196
ALP (IU/L)	125.4 ± 108.5	140.6 ± 128.8	108.9 ± 78.0	0.012

Tokyo guideline grade				0.005
I	166 (58.9%)	73 (49.7%)	93 (68.9%)	
II	105 (37.2%)	67 (45.6%)	38 (28.1%)	
III	11 (3.9%)	7 (4.8%)	4 (3.0%)	
Biliary drainage				0.002
PTGBD	83 (29.4%)	52 (35.4%)	31 (23.0%)	
PTBD	11 (3.9%)	8 (5.4%)	3 (2.2%)	
ERBD	52 (18.4%)	40 (27.2%)	12 (8.9%)	

ASA indicates American Society of Aneshesiologists; CBD, Common bile duct; WBC, White blood cell; CRP, C-reactive protein; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; PTGBD, Percutaneous transhepatic gallbladder drainage; PTBD, Percutaneous transhepatic biliary drainage; ERBD, Endoscopic retrograde biliary drainage.

Table 2. Perioperative outcomes

Variables	Total (n = 282)	Culture (+) (n = 147)	Culture (−) (n = 135)	P value
Intraoperative findings				
Operation time (mins)	47.5 ± 22.9	51.1 ± 25.5	43.7 ± 19.0	0.007
Biliary tract injury	5 (1.8%)	5 (3.4%)	0 (0.0%)	0.031
Bile leakage	43 (15.2%)	32 (21.8%)	11 (8.1%)	0.001
Drainage insertion	144 (51.1%)	93 (63.3%)	51 (37.8%)	0.001
Postoperative outcomes				
Fever	224 (79.4%)	119 (81.0%)	105 (77.8%)	0.510
Duration (days)	2.1 ± 1.8	2.3 ± 2.0	1.9 ± 1.5	0.077
Antibiotics	207 (73.4%)	112 (76.2%)	85 (63.0%)	0.001
Duration (days)	4.0 ± 3.4	4.3 ± 2.9	3.7 ± 3.9	0.254
Lab findings				
WBC (/μL)	9441.9 ± 3623.7	9672.8 ± 3550.4	9026.2 ± 3752.2	0.313
CRP (mg/L)	9.9 ± 7.9	11.5 ± 8.0	7.1 ± 6.9	0.002
Total bilirubin (mg/dL)	1.0 ± 0.7	1.0 ± 0.6	1.1 ± 1.0	0.817
AST (IU/L)	52.1 ± 60.9	52.1 ± 70.4	52.1 ± 39.8	0.999
ALP (IU/L)	118.6 ± 119.6	118.9 ± 84.8	118.1 ± 164.9	0.971
Hospital stay (days)	4.3 ± 7.3	4.8 ± 9.0	3.7 ± 4.7	0.214
Complication (CD grade ≥3a)	36 (12.8%)	17 (11.6%)	19 (14.1%)	0.528

WBC indicates White blood cell; CRP, C-reactive protein; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; CD, Clavien Dindo.

Table 3. Antibiotic susceptibilities of the four most commonly isolated microorganisms

Antibiotics	Escherichia (n=53)	Klebsiella (n=28)	Enterobacter (n=18)	Enterococcus (n=37)
Amikacin	51 (96.2%)	28 (100%)	18 (100%)	-
Ampicillin	19 (35.8%)	0 (0.0%)	5 (27.8%)	25 (67.6%)
Aztreonam	40 (75.5%)	27 (96.4%)	14 (77.8%)	-
Cefazolin	25 (47.2%)	22 (78.6%)	2 (11.1%)	-
Cefotetan	51 (96.2%)	28 (100%)	13 (72.2%)	-
Cefotaxime	37 (69.8%)	27 (96.4%)	12 (66.7%)	-
Ceftazidime	41 (77.4%)	27 (96.4%)	14 (77.8%)	-
Ciprofloxacin	32 (60.4%)	25 (89.3%)	17 (94.4%)	14 (37.8%)
Imipenem	53 (100%)	28 (100%)	18 (100%)	-
Vancomycin	-	-	-	31 (83.8%)
Teicoplanin	-	-	-	31 (83.8%)

Table 4. Clinical findings of patients infected with specific microorganisms

Variables	Escherichia (n=53)	Klebsiella (n=28)	Enterobacter (n=18)	Enterococcus (n=37)	P value
Age (years)	70.7 ± 10.7	71.1 ± 10.1	67.5 ± 10.0	69.5 ± 14.2	0.708
Male sex	28 (52.8%)	19 (67.9%)	15 (83.3%)	25 (67.6%)	0.103
Symptoms					
Abdominal pain	49 (92.5%)	27 (96.4%)	18 (100%)	31 (83.8%)	0.219
Fever (≥38.0°C)	19 (35.8%)	14 (50.0%)	4 (22.2%)	20 (54.1%)	0.084
Radiologic findings					
Gallbladder stone	38 (71.7%)	21 (75.0%)	17 (94.4%)	33 (89.2%)	0.017
CBD stone & dilatation	6 (11.3%)	2 (7.1%)	6 (33.3%)	19 (51.4%)	0.001
Cholangiohepatitis	8 (15.1%)	2 (7.1%)	2 (11.1%)	16 (43.2%)	0.001
Lab findings					
WBC (/μL)	14972.3 ± 6873.1	14288.6 ± 5034.3	13387.8 ± 6980.4	15510.8 ± 7157.4	0.697
Total bilirubin (mg/dL)	1.7 ± 1.7	2.5 ± 2.0	2.5 ± 2.9	4.2 ± 3.4	0.001
AST (IU/L)	116.5 ± 241.1	78.4 ± 128.6	56.5 ± 87.0	205.5 ± 251.7	0.037
ALT (IU/L)	56.9 ± 93.7	61.0 ± 100.0	78.9 ± 175.7	170.4 ± 191.3	0.001
ALP (IU/L)	136.4 ± 132.8	114.0 ± 69.7	96.5 ± 58.2	221.2 ± 185.3	0.002
Tokyo guideline grade					0.232
I	20 (37.7%)	12 (42.9%)	12 (66.7%)	19 (51.4%)	
II	32 (58.5%)	13 (46.4%)	6 (33.3%)	15 (40.5%)	
III	2 (3.8%)	3 (10.7%)	0 (0.0%)	3 (8.1%)	
Biliary drainage	24 (45.3%)	11 (39.3%)	10 (55.6%)	30 (81.1%)	0.002

CBD indicates Common bile duct; WBC, White blood cell; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase.

Table 5. Clinical findings of patients infected with and without ESBL-producing microorganisms

Variables	ESBL (+) (n=25)	ESBL (-) (n=74)	P value
Age (years)	71.6 ± 8.4	68.1 ± 12.3	0.197
Male sex	12 (48.0%)	47 (63.5%)	0.172
Symptoms			
Abdominal pain	24 (96.0%)	72 (97.3%)	0.744
Fever (≥38.0°C)	11 (44.0%)	22 (29.7%)	0.191
Radiologic findings			
Gallbladder stone	20 (80.0%)	55 (74.3%)	0.567
CBD stone & dilatation	9 (36.0%)	5 (6.8%)	0.001
Cholangiohepatitis	7 (28.0%)	3 (4.1%)	0.001
Lab findings			
WBC (/μL)	14066.8 ± 8350.1	14127.0 ± 5604.5	0.973
Total bilirubin (mg/dL)	2.1 ± 1.8	1.8 ± 1.8	0.440
AST (IU/L)	193.3 ± 290.1	68.9 ± 200.2	0.019
ALT (IU/L)	138.8 ± 186.0	38.6 ± 91.2	0.015
ALP (IU/L)	144.0 ± 141.7	98.6 ± 61.1	0.132
Tokyo guideline grade			0.487
I	12 (48.0%)	35 (47.3%)	
II	13 (52.0%)	35 (47.3%)	
III	0 (0.0%)	4 (5.4%)	
Biliary drainage	16 (64.0%)	24 (32.4%)	0.005

ESBL indicates Extended-spectrum beta-lactamase; CBD, Common bile duct; WBC, White blood cell; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase.

Table 6. Microorganisms according to the type of preoperative biliary drainage

Variables	Upfront surgery (n=162)	PTGBD (n=83)	PTBD (n=11)	ERBD (n=52)	P value
Culture results					0.001
Culture (+)	68 (42.0%)	52 (62.7%)	8 (72.7%)	40 (76.9%)	
Culture (-)	94 (58.0%)	31 (37.3%)	3 (27.3%)	12 (23.1%)	
Gram staining					0.001
Gram (-)	59/68 (86.8%)	38/52 (73.1%)	4/8 (50.0%)	14/40 (35.0%)	
Escherichia	29 (42.6%)	17 (32.7%)	1 (12.5%)	6 (15.0%)	
Klebsiella	17 (25.0%)	9 (17.3%)	1 (12.5%)	0 (0.0%)	
Enterobacter	8 (11.7%)	7 (13.5%)	0 (0.0%)	4 (10.0%)	
Gram (+)	9/68 (13.2%)	14/52 (26.9%)	4/8 (50.0%)	21/40 (52.5%)	
ESBL-producing microorganisms	9 (13.2%)	6 (11.5%)	2 (25.0%)	9 (22.5%)	0.002

PTGBD indicates Percutaneous transhepatic gallbladder drainage; PTBD, Percutaneous transhepatic biliary drainage; ERBD, Endoscopic retrograde biliary drainage; ESBL, Extended-spectrum beta-lactamase.

Supplementary table 1. The species of microorganism in patients with polymicrobial infection

Patient	Microorganism 1	Microorganism 2	Microorganism 3
1	Escherichia	Enterococcus	-
2	Escherichia	Enterococcus	-
3	Escherichia	Klebsiella	-
4	Escherichia	Klebsiella	-
5	Escherichia	Enterobacter	-
6	Escherichia	Citrobacter	Enterococcus
7	Klebsiella	staphylococcus	-
8	Enterobacter	Streptococcus	-
9	Enterobacter	Candida	-
10	Enterococcus	Klebsiella	-
11	Enterococcus	Streptococcus	-
12	Enterococcus	Pseudomonas	Escherichia
13	Enterococcus	Raoultella	-

Figure 1. Flow chart showing an overview of the study enrollment and exclusions

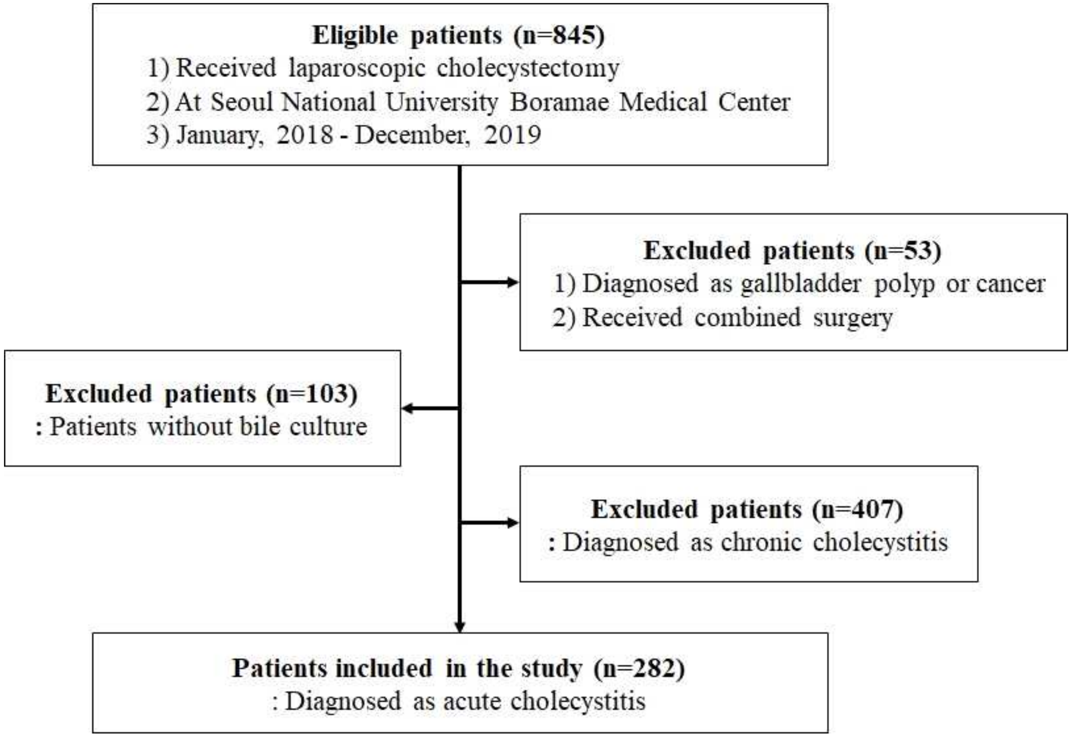


Figure 2. Microorganisms isolated from culture-positive patients

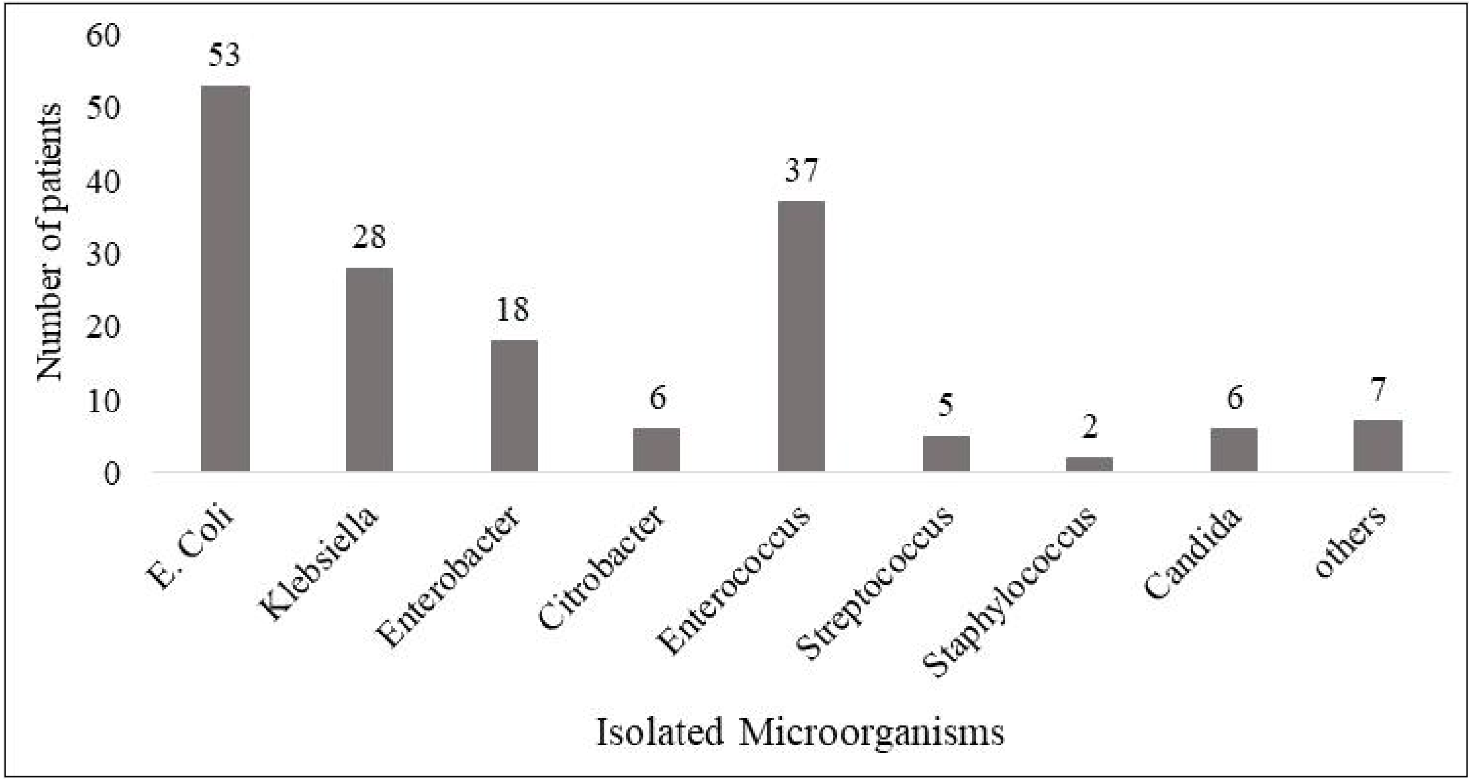
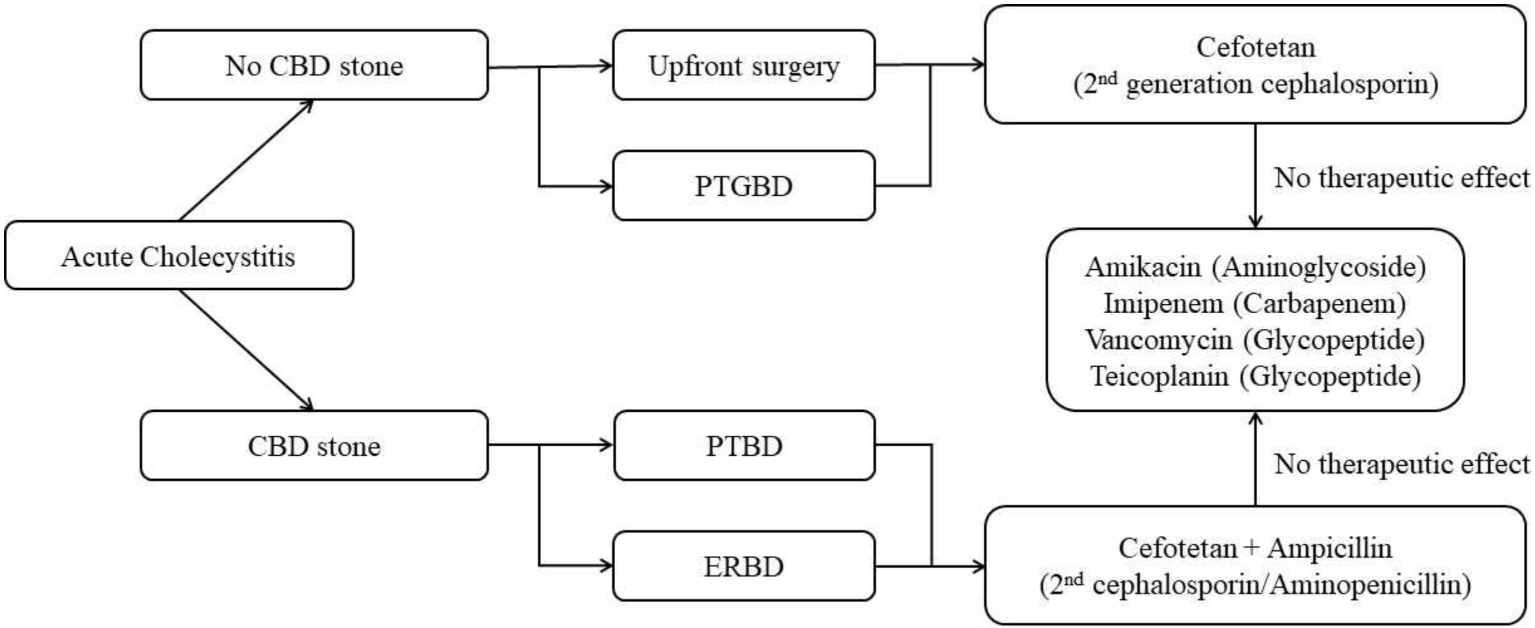


Figure 3. Selection of empirical antibiotics according to the type of preoperative biliary drainage



국문초록

급성 담낭염 환자에서 담즙 배양의 미생물학 및 항생제 감수성에 따른 경험적 항생제 선택에 대한 연구

배경: 급성 담낭염 환자에서 세균 감염은 흔하게 발생하며, 경험적 항생제 선택은 중요하다. 따라서 급성 담낭염 환자에서 확인된 미생물 및 항생제 감수성을 토대로 환자의 수술 전 임상 양상을 비교하여 더 적절한 경험적 항생제를 제시하고자 본 연구를 시행하였다.

방법: 본 연구는 2018년부터 2019년까지 서울대학교 보라매병원에서 급성 담낭염으로 수술을 받은 환자를 대상으로 의무기록 검토를 통해 진행되었다. 담즙 배양 및 항생제 감수성 검사가 시행되었고, 환자에 따른 임상 양상을 조사하였다.

결과: 분석에 포함된 282명의 환자 중 배양-양성은 147명, 배양-음성은 135명으로 확인되었다. 가장 흔하게 배양된 균주는 *Escherichia* (n=53, 32.7%), *Enterococcus* (n=37, 22.8%), *Klebsiella* (n=28, 17.3%), 그리고 *Enterobacter* (n=18, 11.1%) 순이었다. 그람 음성균의 경우, 2세대 세팔로스포린계 항생제(cefotetan: 96.2%)가 3세대 세팔로스포린계 항생제(cefotaxime: 69.8%)보다 더 효과적인 것으로 나타났다. *Enterococcus* 균주의 경우, 반코마이신과 테이코플라닌(83.8%)이 가장 효과적인 항생제로 확인되었다. 또한, 다른 균주에 감염된 환자에 비해 *Enterococcus*에 감염된 환자에서 총담관결석이 있거나 (51.4%, p=0.001), 담도배액술을 시행받은 경우가 더 많았고 (81.1%, p=0.002), 높은 간효소 수치를 보였다 (p=0.001). ESBL 생산 균주를 가진 환자의 경우, 다른 균주에 감염된 환자에 비해 총담관결석이 있거나 (36.0% vs. 6.8%, p=0.001), 담도배액술을 시행받은 경우가 더 많았다 (64.0% vs. 32.4%, p=0.005).

결론: 급성 담낭염 환자에서 수술 전 임상 양상은 감염된 세균의 종류와 관련이 있다. 또한, 적절한 경험적 항생제를 선택하기 위해서는 지역 사회의 환자를 대상으로 주기적인 항생제 감수성 검사를 시행해야 한다.

주요어 : 담낭염, 항생제, 미생물학, 항생제 감수성 검사
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