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ORIGINAL ARTICLE

급성담관염의 원인균주와 항생제감수성의 시간흐름에 따른 변화

권정석, 한지민, 김태원, 오지혜, 권현희, 정진태, 권중구, 김은영, 김호각
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Changes in Causative Pathogens of Acute Cholangitis and Their Antimicrobial Susceptibility over a Period of 6 Years

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Background/Aims: We evaluated changes of causative pathogen in acute cholangitis and their antimicrobial susceptibility over six years and differences between community-acquired and hospital-acquired acute cholangitis at our institution.
Methods: Medical records of 1,596 patients with acute cholangitis and biliary drainage between August 2006 and August 2012 were reviewed retrospectively. Cases were divided according to time: period 1 (August 2006-December 2008, n=645, 40.4%), period 2 (January 2009-August 2012, n=951, 59.6%). Cases were divided according to community-acquired cholangitis (n=1,397, 87.5%) and hospital-acquired cholangitis (n=199, 12.5%). Causative pathogens and antimicrobial susceptibility were investigated in each group.
Results: Causative pathogen was isolated from bile culture in 1,520 out of 1,596 cases (95.2%). The three most frequently isolated Gram-negative bacteria were extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (n=485, 30.4%), *E. coli* (n=237, 13.2%), and *Citrobacter freundii* (n=110, 6.9%). Between periods 1 and 2, prevalence of ESBL-producing *E. coli* and *Klebsiella pneumoniae* did not show significant change (36.7% *vs.* 32.1%, p=0.073; 6.6% *vs.* 6.2%, p=0.732). *C. freundii* showed a significant increase from period 1 to period 2 (1.7% *vs.* 13.2%, p=0.000). In both time periods, imipenem was the antimicrobial agent showing the highest rate of susceptibility (93.3% *vs.* 93.9%, p=0.783). Higher prevalence of ESBL-producing *E. coli* and *C. freundii* was observed in the hospital-acquired cholangitis group (52.1% *vs.* 31.2%, p=0.000; 15.9% *vs.* 7.3%, p=0.001).
Conclusions: The most common causative pathogen of acute cholangitis was ESBL-producing *E. coli*. Prevalence of *C. freundii* increased over the time period. Imipenem should be reserved as an alternative for resistant pathogens. **(Korean J Gastroenterol 2014;63:299-307)**

Key Words: Cholangitis; Microbial sensitivity tests; Antimicrobial drug resistance

INTRODUCTION

Bactobilia is defined as the presence of microbial pathogens in bile and acute cholangitis is caused by bacterial infection in the setting of biliary obstruction.[1] The most common predisposing factors of acute cholangitis are common bile duct stones, tumors of the pancreatobiliary system, and benign biliary stricture.[2,3] In its most severe form, acute cholangitis may result in biliary sepsis or even multi-organ failure. Consequently, morbidity rate and mortality rate of patients

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with acute cholangitis have increased.[4] Therefore, in addition to biliary drainage, empirical therapy with a broad-spectrum antimicrobial agent is warranted in management of acute cholangitis. However, recent studies have reported that isolates from acute cholangitis have changed due to increased use of biliary stents and prior exposure to antimicrobial agents.[5,6] Rate of infection with extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria isolates has increased in both community-acquired infections and in hospital-acquired infections.[7,8] Thus, knowledge of prevalence and antimicrobial susceptibility of the institution is essential to administration of the most effective empirical antimicrobial therapy.

In this study, we investigated the etiology of acute cholangitis and causative pathogens isolated from blood and bile culture of patients with acute cholangitis over a time period of six years. In addition, we also investigated changes of causative pathogens and their antimicrobial susceptibility over the time period and differences in causative pathogens and their antimicrobial susceptibility between community-acquired and hospital-acquired acute cholangitis.

SUBJECTS AND METHODS

Patients with acute cholangitis who received biliary drainage at a single university medical center between August 2006 and August 2012 were included in this study. Medical records of these patients were reviewed retrospectively. Acute cholangitis was diagnosed when more than one of the following was found: 1) purulent bile in gross appearance, 2) leukocytes in bile ≥50 per high power field, 3) positive growth in bile culture. Biliary drainage included percutaneous transhepatic biliary drainage, percutaneous transhepatic gallbladder drainage, or ERCP. Bile was aspirated using sterile syringes through sterile biliary drainage catheters. Hospital-acquired cholangitis was defined as follows: no evidence of cholangitis before the first biliary drainage procedure and development of acute cholangitis after biliary drainage. Data on demographics, causes of acute cholangitis, laboratory findings, results of blood and/or bile cultures, causative pathogens, their antimicrobial suscepti-

Table 1. Baseline Characteristics of the Study Subjects (n=1,596)

Characteristic	Data
Age (yr)	68.3±13.8
Male	905 (56.7)
ALT (IU/L)	179.6±205.9
AST (IU/L)	185.9±239.8
ALP (IU/L)	594.3±510.8
GGT (IU/L)	395.1±439.6
Bilirubin (mg/dL)	7.1±32.9
CRP (mg/L)	59.76±76.8
Positive growth in blood	116 (14.1)
Positive growth in bile	1,513 (95.2)
Positive growth in both blood and bile	116 (7.6)
Concordant growth between blood and bile cultures	90 (77.6)
Causes of acute cholangitis	
CBD+IHD stone	1,053 (66)
Malignant tumor	
Cholangiocarcinoma	215 (13.5)
Pancreatic head cancer	59 (3.7)
GB cancer	35 (2.2)
AOV tumor	59 (3.7)
Metastatic cancer	16 (1.0)
Benign biliary stricture	29 (1.8)
Others	130 (8.1)

Values are presented as mean±SD or n (%).
CBD, common bile duct; IHD, intrahepatic bile duct; GB, gallbladder; AOV, ampulla of Vater.

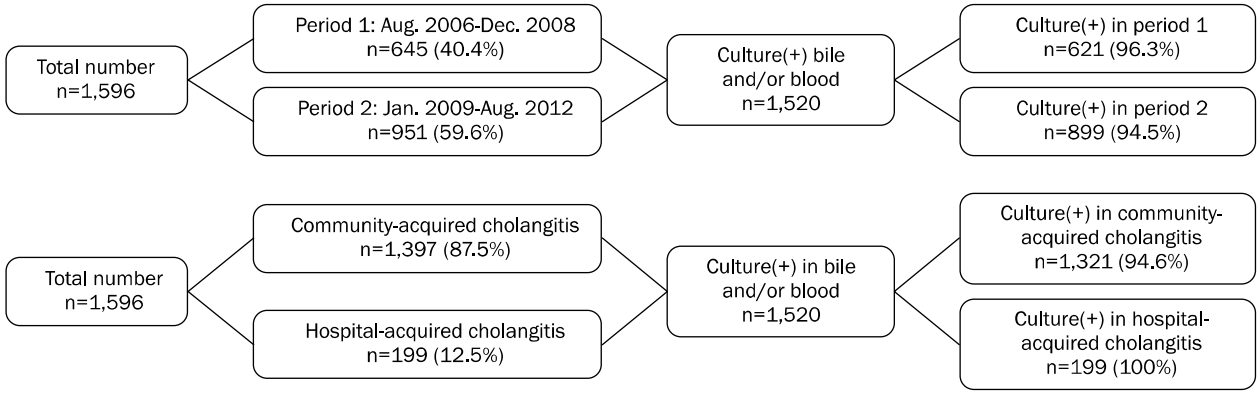

Fig. 1. Study flow. Patients with acute cholangitis and biliary drainage were divided into groups according to time period and setting.

bility, implemented treatment, and clinical outcome were collected in each case.

A total of 1,596 cases were included for analysis (Table 1). There were 691 females (43.3%) and 905 males (56.7%). Mean age of the patients was 68.3±13.8 years. Blood and/or bile culture were obtained from 1,520 cases. For analysis, the cases were classified according to time period and setting (Fig. 1). The cases were arbitrarily divided according to two time periods: period 1 (August 2006-December 2008; n=645, 40.4%) and period 2 (January 2009-August 2012; n=951, 59.6%). Causative pathogens and their antimicrobial susceptibility were evaluated in each group. The cases were then divided according to community-acquired cholangitis (n=1,397, 87.5%) and hospital-acquired cholangitis (n=199, 12.5%). Again, causative pathogens and their antimicrobial susceptibility were evaluated in each group.

Statistical analysis was performed using SPSS 19.0 software for Windows (IBM, Armonk, NY, USA). Values were presented as mean±SD. For comparison, independent t-test was used for continuous variables and chi-square test for categorical variables. p-value <0.05 was considered statistically significant.

Table 2. Comparison of Baseline Characteristics between Period 1 and 2

Characteristic	Period 1 (n=645)	Period 2 (n=951)	p-value
Age (yr)	69.3±13.8	68.62±13.8	0.914
Male	386 (59.8)	522 (54.9)	0.051
ALT (IU/L)	184.7±213.6	175.8±200.0	0.082
AST (IU/L)	178.1±214.9	191.0±254.7	0.144
ALP (IU/L)	593.1±505.5	598.2±521.2	0.803
GGT (IU/L)	394.2±381.0	396.7±475.2	0.458
Bilirubin (mg/dL)	8.64±45.52	6.04±19.8	0.016
CRP (mg/L)	59.3±68.6	60.2±80.9	0.175
Positive growth in blood	66 (19)	50 (10.4)	0.001
Positive growth in bile	621 (96.3)	899 (94.5)	0.120
Causes of acute cholangitis			
CBD+IHD stone	417 (64.7)	636 (66.9)	0.361
Malignant tumor			
Cholangiocarcinoma	87 (13.5)	128 (13.5)	1.000
Pancreatic head cancer	20 (3.1)	39 (4.1)	0.345
GB cancer	14 (2.2)	21 (2.2)	1.000
AOV tumor	22 (3.4)	37 (3.9)	0.686
Metastatic cancer	10 (1.6)	6 (0.6)	0.078
Benign biliary stricture	13 (2.0)	16 (1.7)	0.703
Others	62 (9.6)	68 (7.2)	0.111

Values are presented as mean±SD or n (%).
CBD, common bile duct; IHD, intrahepatic bile duct; GB, gallbladder; AOV, ampulla of Vater.

RESULTS

1. Causes of acute cholangitis and biliary drainage

Causes of acute cholangitis and biliary drainage are described in Table 2. The most common cause of acute cholangitis and biliary drainage was biliary stone (n=1,053, 66%), followed by cholangiocarcinoma (n=215, 13.5%), pancreatic head cancer (n=59, 3.7%), and ampulla of Vater tumor (n=59, 3.7%). No statistically significant difference in causes was observed between period 1 and period 2.

2. Changes of causative pathogens and their susceptibility over the time period

Data from comparison of baseline characteristics of the patients between two time periods are shown in Table 2. Although total bilirubin was higher in period 1 and frequency of bacteremia was higher in period 2, no statistically significant differences in other parameters were observed between the two time periods.

Changes of causative pathogens and their antimicrobial susceptibility over the time period of six years are described in Table 3. In both time periods, ESBL-producing *Escherichia coli* was the most common causative pathogen, followed by *E. coli* not producing ESBL, *Citrobacter freundii*, and *Klebsiella pneumoniae* not producing ESBL. When compared with period 1, proportion of *E. coli* not producing ESBL showed a significant decrease in period 2 (19.4% *vs.* 14.6%, p=0.02). In addition, proportions of ESBL-producing *E. coli* (36.7% *vs.* 32.1%, p=0.073) and ESBL-producing *K. pneumoniae* (6.6% *vs.* 6.2%, p=0.732) did not show a significant change over the time period. On the other hand, proportion of *C. freundii* showed a significant increase in period 2 when compared with that in period 1 (1.7% *vs.* 13.2%, p=0.000). However, proportions of *Pseudomonas aeruginosa* (7.1% *vs.* 4.3%, p=0.024) and *Acinetobacter baumanii* (3.7% *vs.* 1.9%, p=0.036) showed a significant decrease over the time period.

Changes in antimicrobial susceptibility over the time period are described in Table 3. No statistically significant change in susceptibility for amikacin (80.9% *vs.* 90%, p=0.000) and imipenem (93.3% *vs.* 93.9%, p=0.783) was observed between period 1 and period 2. Although no statically significant difference was observed between period 1 and period 2, penicillin, third generation cephalosporins, fourth generation cephalosporins, and quinolones showed low susceptibility of around 50%.

Table 3. Changes in Causative Pathogens and Their Antimicrobial Susceptibility over the Time Period of Six Years

Variable	Culture(+) in period 1 (n=621)	Culture(+) in period 2 (n=899)	p-value	Culture(+) in both (n=1,520)
Causative pathogen, n (%)				
Gram-negative				
E. coli	112 (19.4)	125 (14.6)	0.020	211 (13.2)
ESBL-producing *E. coli*	210 (36.7)	362 (32.1)	0.073	485 (30.4)
K. pneumoniae	37 (6.4)	65 (7.6)	0.405	99 (6.2)
ESBL-producing *K. pneumoniae*	38 (6.6)	53 (6.2)	0.732	91 (5.7)
Pseudomonas aeruginosa	41 (7.1)	37 (4.3)	0.024	72 (4.5)
Citrobacter freundii	10 (1.7)	100 (13.2)	0.000	110 (6.9)
Enterobacter cloacae	36 (6.3)	50 (6.2)	0.725	86 (5.4)
Acinetobacter baumanii	21 (3.7)	16 (1.9)	0.036	37 (2.3)
Susceptible antimicrobial agent, n (%)				
Aminoglycoside				
Amikacin	293 (80.9)	515 (90)	0.000	
Quinolone				
Ciprofloxacin	185 (52.4)	204 (49.4)	0.426	
Beta-lactams				
Ampicillin	56 (18.1)	59 (12)	0.022	
Piperacillin-tazobactam	219 (65.6)	348 (68.1)	0.455	
Cefotetan	79 (55.6)	172 (74.8)	0.000	
Ceftazidime	130 (53.9)	277 (49.7)	0.281	
Cefepime	232 (64.3)	358 (62.3)	0.578	
Imipenem	336 (93.3)	540 (93.9)	0.783	

ESBL, extended-spectrum beta-lactamase; *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*.

Table 4. Comparison of Baseline Characteristics between Community-acquired Cholangitis and Hospital-acquired Cholangitis

	Community-acquired cholangitis (n=1,397)	Hospital-acquired cholangitis (n=199)	p-value
Age (yr)	68.6±13.9	70.9±12.6	0.230
Male	799 (57.2)	109 (54.8)	0.541
ALT (IU/L)	181.6±206.0	125.1±188.6	0.133
AST (IU/L)	187.3±241.4	145.8±176.2	0.198
ALP (IU/L)	579.2±514.9	568.2±515.6	0.432
GGT (IU/L)	399.5±445.7	300.6±221.2	0.023
Bilirubin (mg/dL)	7.1±33.4	4.6±7.1	0.564
CRP (mg/L)	60.2±77.1	50.4±66.9	0.119
Positive growth in blood	112 (14.1)	47 (11.8)	1.000
Positive growth in bile	1,321 (94.6)	199 (100)	0.000
Causes of acute cholangitis			
CBD+IHD stone	924 (66.3)	129 (64.2)	0.577
Malignant tumor			
Cholangiocarcinoma	182 (12.9)	33 (16.4)	0.183
Pancreatic head cancer	52 (3.7)	7 (3.5)	1.000
GB cancer	29 (2.1)	6 (3.0)	0.436
AOV tumor	54 (3.9)	5 (2.5)	0.425
Metastatic cancer	13 (0.9)	3 (1.5)	0.430
Benign biliary stricture	26 (1.9)	3 (1.5)	0.703
Others	117 (8.2)	13 (7.5)	0.784

Values are presented as mean±SD or n (%).
CBD, common bile duct; IHD, intrahepatic bile duct; GB, gallbladder; AOV, ampulla of Vater.

Table 5. Comparison of Causative Pathogens and Their Antimicrobial Susceptibility between Community-acquired Cholangitis and Hospital-acquired Cholangitis

	Culture(+) in community-acquired cholangitis (n=1,321)	Culture(+) in hospital-acquired cholangitis (n=199)	p-value	Culture(+) in both (n=1,520)
Causative pathogen, n (%)				
Gram-negative				
E. coli	226 (18.2)	11 (5.8)	0.000	211 (13.2)
ESBL-producing *E. coli*	386 (31.2)	99 (52.1)	0.000	485 (30.4)
K. pneumoniae	100 (8.0)	2 (1.1)	0.000	99 (6.2)
ESBL-producing *K. pneumoniae*	77 (6.6)	14 (7.9)	0.546	91 (5.7)
Pseudomonas aeruginosa	68 (5.5)	10 (5.3)	1.000	72 (4.5)
Citrobacter freundii	84 (7.3)	26 (15.9)	0.001	110 (6.9)
Enterobacter cloacae	77 (6.6)	9 (5.0)	0.424	86 (5.4)
Acinetobacter baumanii	30 (2.4)	7 (3.8)	0.308	37 (2.3)
Susceptible antimicrobial agent, n (%)				
Aminoglycoside				
Amikacin	735 (86.9)	73 (83)	0.708	
Quinolone				
Ciprofloxacin	368 (52.7)	21 (30.9)	0.001	
Beta-lactams				
Ampicillin	112 (15.5)	3 (3.8)	0.003	
Piperacillin-tazobactam	529 (69.2)	38 (47.5)	0.000	
Cefotetan	232 (68.6)	19 (55.9)	0.178	
Ceftazidime	394 (55.3)	13 (15.1)	0.000	
Cefepime	556 (65.7)	34 (37.8)	0.000	
Imipenem	79 (94.1)	82 (90.1)	0.168	

ESBL, extended-spectrum beta-lactamase; *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae.*

3. Comparison of causative pathogens and their antimicrobial susceptibility between community-acquired cholangitis and hospital-acquired cholangitis

Data from comparison of baseline characteristics of the patients between community-acquired cholangitis and hospital-acquired cholangitis are shown in Table 4. Level of GGT was higher in community-acquired cholangitis (399.5±445.7 *vs.* 300.6±221.2, p=0.023). Age of patients did not differ between the two groups (70.9±12.6 *vs.* 68.6±13.9, p=0.23). Higher rate of positive growth in bile culture was observed for patients with hospital-acquired cholangitis (100% *vs.* 94.6%, p=0.000).

Data from comparison of causative pathogens and their antimicrobial susceptibility between community-acquired cholangitis and hospital-acquired cholangitis are shown in Table 5. ESBL-producing *E. coli* was more common in hospital-acquired cholangitis than in community-acquired cholangitis (52.1% *vs.* 31.2%, p=0.000). In addition, *C. freundii* was more common in hospital-acquired cholangitis than in community-acquired cholangitis (15.9% *vs.* 7.3%, p=0.001). However, *E. coli* not producing ESBL (18.2% *vs.* 5.8%, p=0.000) and *K. pneumoniae* not producing ESBL (1.1% *vs.* 8.0%, p=0.000) were less common in hospital-acquired cholangitis than in community-acquired cholangitis. Data from comparison of antimicrobial susceptibility between community-acquired cholangitis and hospital-acquired chol-

Table 6. Comparison of Antimicrobial Susceptibility of Extended-spectrum Beta-lactamase-producing *Escherichia coli* between Period 1 and 2

Antimicrobial agent, n (%)	ESBL(+) *E. coli* in period 1 (n=210)	ESBL(+) *E. coli* in period 2 (n=362)	p-value
Aminoglycoside			
Amikacin	183 (86.3)	218 (80.1)	0.089
Quinolone			
Ciprofloxacin	17 (8.1)	11 (5.0)	0.241
Beta-lactams			
Ampicillin	3 (1.4)	3 (1.1)	1.000
Piperacillin-tazobactam	181 (85.8)	169 (67.6)	0.000
Cefotetan	88 (90.7)	164 (92.7)	0.644
Ceftriaxone	2 (1.0)	4 (2.4)	0.421
Cefepime	4 (1.9)	8 (2.9)	0.563
Imipenem	208 (99.5)	272 (99.6)	1.000

ESBL, extended-spectrum beta-lactamase; *E. coli*, *Escherichia coli*.

Table 7. Comparison of Antimicrobial Susceptibility of Extended-spectrum Beta-lactamase-producing *E. coli* between Community-acquired Cholangitis and Hospital-acquired Cholangitis

Antimicrobial agent, n (%)	ESBL(+) *E. coli* in community-acquired cholangitis (n=386)	ESBL(+) *E. coli* in hospital-acquired cholangitis (n=99)	p-value
Aminoglycoside			
Amikacin	318 (82.6)	83 (83.8)	0.881
Quinolone			
Ciprofloxacin	25 (7.3)	3 (3.4)	0.231
Beta-lactams			
Ampicillin	6 (1.6)	0 (0.0)	0.353
Piperacillin-tazobactam	284 (77.2)	66 (71.0)	0.223
Cefotetan	187 (91.7)	65 (92.9)	1.000
Ceftriaxone	5 (1.8)	1 (1.2)	1.000
Cefepime	11 (2.9)	1 (1.0)	0.474
Imipenem	382 (99.7)	98 (99.0)	0.369

ESBL, extended-spectrum beta-lactamase; *E. coli*, *Escherichia coli*.

angitis are shown in Table 5. Amikacin and imipenem were the two antimicrobial agents showing the highest susceptibility in both groups. In hospital-acquired cholangitis, ampicillin, piperacillin-tazobactam, cephalosporins, and quinolones showed lower susceptibility than in community-acquired cholangitis.

4. Changes in antimicrobial susceptibility of ESBL-producing *E. coli*

Antimicrobial susceptibility of ESBL-producing *E. coli* was compared between period 1 and period 2 (Table 6), and its antimicrobial susceptibility was compared between community-acquired cholangitis and hospital-acquired cholangitis (Table 7). Regardless of time period and setting, amikacin and imipenem showed the highest susceptibility. Piperacillin-tazobactam showed decreased susceptibility in period 2 (85.8% *vs.* 76.6%, p=0.000), but similar susceptibility in community-acquired cholangitis and hospital-acquired cholangitis (77.2% *vs.* 71.0%, p=0.223).

DISCUSSION

Acute cholangitis is a medical emergency and can be life-threatening without appropriate treatment. In normal biliary tract, bile is sterile. However, bacterial colonization of bile occurs through ascension from the duodenum or translocation from the portal vein.[1,9] Then, biliary obstruction results in increased intraductal pressure and reflux of infected bile into blood and lymphatics.[1,10] The most common cause of biliary obstruction and resultant acute cholangitis is common bile duct stone.[11] With an increase in number of interventions, such as surgery, percutaneous transhepatic biliary drainage, and ERCP, the rate of acute cholangitis in patients with malignant biliary obstruction has shown a gradual increase.[3,12] Two studies from academic centers reported that bacteria were isolated from 31.2% and 80% of blood and from 71.7% and 96.3% of bile obtained from patients with acute cholangitis.[13,14] Bacteria isolated from blood are similar to bacteria isolated from bile, therefore, isolation from bile culture can provide solid evidence for selection of a susceptible antimicrobial agent.[15,16] In this study, bacteria were isolated from 14.1% of blood cultures, and from 95.2% of bile cultures. Bacteria were isolated from both blood and bile cultures in 7.6%. In other studies of acute cholangitis, the common causative pathogen isolated from blood and/or bile cultures was *E. coli*.[14,17-22] Similar to findings of previous studies, in this study, Gram-negative bacteria were predominantly found in 94% of patients with acute cholangitis. In addition, the most common Gram-negative pathogens were *E. coli*. These results are similar to those from previous studies.[22-24] A study from Japan reported that Gram-negative aerobic bacteria was most frequently isolated from patients with biliary infection.[23] Bacteriologic studies of bile and blood cultures from Hong Kong showed that Gram-negative bacteria, particularly *E. coli*, were found most frequently.[17] A similar study from Poland reported that Gram-negative bacteria were isolated in 68.1% of bile cultures and the most predominant Gram-negative pathogen was *E. coli*.[24] In this study, the causative pathogen for more than one-third of cases was ESBL-producing *E. coli* isolated from blood or bile, and the causative pathogen for 6.3% of cases was ESBL-producing *K. pneumoniae* isolated from blood or bile. In addition, with regard to change of time period and antimicrobial susceptibility, ESBL-producing *E. coli* was the most common bacteria in both period 1 and period 2, but did not show a significant increase over time. Unlike previous studies[13,14,17,21,23] showing frequency of *C. freundii* to be less than 5%, frequency of *C. freundii* increased in this study. Previous studies have reported an increase in prevalence of resistant bacteria over time, change in their antimicrobial susceptibility, and difference according to region and country in which studies are

conducted.[18,20,25-27]

Tokyo Guidelines suggested that in cases of light acute cholangitis (grade I), use of a beta-lactam/beta-lactamase inhibitor such as piperacillin-tazobactam or ampicillin-sulbactam as monotherapy is adequate.[28] In cases of moderate (grade II) and severe (grade III) acute cholangitis, the recommended initial empiric antimicrobial agents are broad-spectrum beta-lactam/beta-lactamase inhibitors such as piperacillin-tazobactam and ampicillin/sulbactam or third- or fourth-generation cephalosporins with wide antimicrobial spectra such as cefoperazone-sulbactam, ceftriaxone, ceftazidime, and cefepime. To cover anaerobes, metronidazole is additionally recommended.

However, in one study, routine administration of metronidazole did not result in improved clinical outcome of community-acquired acute cholangitis.[22] Depending on the susceptibility patterns, drugs to be selected in the second-line are fluoroquinolones (ciprofloxacin, levofloxacin, and pazufloxacin) and carbapenems (meropenem, imipenem-cilastatin, and doripenem).[29] In this study, imipenem was the antimicrobial agent showing the highest susceptibility in both period 1 and period 2. Imipenem, the most effective drug against Gram-negative pathogens, should remain the last resort, when all other antimicrobial agents are ineffective. Typically, poor outcome is expected in infection by antibiotic-resistant bacteria.[30] According to the results of this study, Gram-negative bacteria showed high resistance to ampicillin, ciprofloxacin, and second, third, and fourth generation cephalosporins in the past and at present. However, cefotetan showed increased susceptibility over the time period. Piperacillin/tazobactam showed susceptibility of 65.6% in period 1; however, it increased to 68.1% in period 2 (p=0.455). The rate of resistance to the cephalosporins disqualifies them as first-line empiric antimicrobial agents against acute cholangitis. However, cephalosporins are still used as first line empiric antimicrobial agents. Susceptibility of amikacin did not show a significant change, with 80.9% in period 1, and 90% in period 2, respectively (p=0.000). Ampicillin and gentamicin were the two empiric antimicrobial agents of choice in the past. With increasing resistance to ampicillin and significant nephrotoxicity caused by aminoglycosides, this combination is not preferred.[31,32] Although aminoglycosides show low concentration in bile, they appear to possess high *in vitro* efficiency against bile pathogens. The issue regarding drug penetration remains controversial. Only a few *ex vivo* studies[32] and clinical *in vivo* observations with inconsistent results have been reported on this topic.[33,34] Aminoglycosides, alone or as part of a combination regimen, were considered effective against acute cholangitis.[35-37] Despite high *in vitro* efficiency against causative pathogen, use of amikacin in treatment of acute cholangitis is controversial due to low concentration in bile. Ciprofloxacin is the only antibiotic actively secreted into bile, especially in cases of cholestasis.[17,38] When bacterial infection spreads beyond bile duct and becomes systemic, it is of critical importance that the antibiotic be effective as soon as possible in order to control bacteremia. Serum concentrations of the studied antibiotics are sufficient to eradicate the infection.[24] However, except for ciprofloxacin, most of the drugs are not actively secreted into the bile, and their bile concentrations are lower than those in blood. However, unlike the other studies, in this study, nearly one half of Gram-negative bacteria showed resistance to ciprofloxacin. Therefore, ciprofloxacin is not an effective empiric antimicrobial agent for acute cholangitis in our institution. In other study, cefepime was very effective despite limited penetration into the bile during cholestasis.[39,40] However, in this study, it was not effective in both period 1 and period 2 (64.3% *vs.* 62.3%, p=0.578). In this study, cefotetan showed increased susceptibility over time (55.6% *vs.* 74.8%, p=0.000). However, high *in vivo* susceptibility of cefotetan does not translate into high *in vitro* susceptibility. Therefore, cefotetan cannot be used in treatment of ESBL-producing *E. coli*.[41,42]

Hospital-acquired infection has been introduced as a risk factor of antibiotic-resistant organism acquisition in previous studies.[27,29] Breach of the enteric-biliary barrier due to stent placement may be an important factor in mixed bacterial colonization of the bile duct.[5] Patients with long hospital stays may have increased chances of exposure to additional antimicrobial agents and to other patients with resistant organisms. As expected, in this study, ESBL-producing *E. coli* and *C. freundii* were more frequently isolated in hospital-acquired cholangitis. According to a study of biliary tract infections conducted in a tertiary referral center, prevalence of ESBL-producing *E. coli* and *Klebsiella* species showed a dramatic increase, from 2.3% to 43.9%, over a 10-year period.[43]

Successful endoscopic biliary drainage with 2-7 days of

antimicrobial therapy until resolution of fever appeared to be safe and effective in a prospective, single-arm, exploratory study of 18 patients with acute cholangitis.[44] However, it is premature to conclude that urgent, adequate biliary drainage with a short course of antimicrobial therapy would decrease antimicrobial resistance without results from a prospective multicenter trial involving a large number of patients. Currently, acute cholangitis is best managed by prompt administration of antimicrobial agent and early and adequate biliary drainage.

This study has some limitations. First, the study was conducted at a single university medical center. Species and prevalence of causative pathogens differ from one institution to another. Therefore, the findings of this study may not be generalized. Conduct of a multicenter study will be necessary in order to overcome this limitation. Second, clinical outcome of patients with acute cholangitis was not assessed. Since administration of the antimicrobial agent according to results of *in vitro* assay of susceptibility does not always guarantee successful clinical outcome, assessment of actual clinical outcome is needed.

In conclusion, the most common causative pathogen of acute cholangitis at our institution was ESBL-producing *E. coli*. Prevalence of *C. freundii* showed a significant increase over a time period of six years. Imipenem is still the most effective antimicrobial agent regardless of time period and setting. However, in order to reduce emergence of pathogens with resistance to carbapenems, they should be used only as an alternative when initial empiric antimicrobial agents such as beta-lactam/beta-lactamase inhibitors or third- or fourth-generation cephalosporins with or without metronidazole fail or microbiologic isolates reveal resistant pathogens. Conduct of further prospective, multicenter studies will be needed in order to make generalizations and to assess clinical significance of changes in causative pathogens and their antimicrobial susceptibility.

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