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Optimizing Perioperative Antimicrobial Therapy for Acute Cholecystitis Using Bile Gram Stain Results

Hiromitsu Maehira, Masayasu Kawasaki, Masao Ogawa, Atsuo Imagawa, Aya Itoh, Naoto Mizumura, Satoshi Okumura, Masao Kameyama

Department of Surgery, Bell Land General Hospital, Osaka, Japan

Objective: We aimed to evaluate whether Gram stain results from smear preparations of bile are useful in determining the optimal perioperative antimicrobial agents.

Summary of background data: Surgical site infections (SSIs) are common complications in emergency cholecystectomy.

Methods: A total of 185 patients who underwent emergency cholecystectomy for acute cholecystitis were enrolled (Bell Land General Hospital Ethic Board approval number 2017-0003). Bile was collected from 121 patients. The Gram stain results from bile smear and culture preparations were evaluated. Furthermore, the antimicrobial resistance was evaluated according to bile Gram stain results.

Results: Bile bacteria were detected in 82 patients (67.8%) with the culture preparation and in 72 patients (59.5%) with the smear preparation. The average rate of correspondence in the Gram stain results between smear and culture preparations was 86.3% among patients with bile bacteria in the smear preparation. Cefepime and meropenem had a low antimicrobial resistance rate for all Gram stain results (<10%). However, the resistance rate of other antimicrobial agents differed according to the Gram stain results.

Conclusions: The Gram stain results from the smear preparation appear to be useful in choosing optimal perioperative antimicrobial agents.

Key words: Acute cholecystitis – Antimicrobial therapy – Gram stain – Infection – Smear preparation

Corresponding author: Hiromitsu Maehira, MD, Department of Surgery, Bell Land General Hospital, 500-3 Naka-ku Higashiyama, Sakai-shi, Osaka 599-8247, Japan.
Tel.: +81 72 234 2001; Fax: +81 72 234 2003; E-mail: hiromitsu0103@gmail.com

In the 2013 Tokyo Guidelines (TG13), the optimal recommended treatment for mild (grade I) and moderate (grade II) acute cholecystitis is early cholecystectomy.[1] Surgical site infections (SSIs) in the superficial wound, fascia, or organ space are common complications of early cholecystectomy for acute cholecystitis. Thus, antimicrobial therapy is recommended to prevent SSIs.[2]

Acute cholecystitis is a typical bactibilia disease, although bile is aseptic.[3,4] Bactibilia is significantly associated with SSIs based on the bacteriologic analyses of bile.[5] A recent increase in antimicrobial resistance rate for enterobacteria has been reported in biliary tract infections.[6,7] Therefore, a bacteriologic bile analysis would aid in optimizing the choice of perioperative antimicrobial agents for the prevention of SSIs. However, an impracticable amount of time is required to obtain the results of a bacterial culture from bile. In contrast, a Gram stain test of a bile smear preparation can provide immediate results and with only a few basic requirements: stain liquid, place to dye the preparation, and microscope. Thus, the Gram stain results of bile smear can be obtained quickly enough to help determine the optimal perioperative antimicrobial agents. However, to our knowledge, no studies have described the antibiogram of bile bacteria according to the results of Gram staining. Moreover, reports describing the risk factors for SSIs in emergent cholecystectomy for acute cholecystitis are lacking.

Therefore, we aimed to compare the Gram stain results obtained using culture and smear preparations and to evaluate the antibiogram of bile bacteria according to Gram stain results. Our overarching goal is to determine whether it is feasible to identify the optimal perioperative antimicrobial agents in emergent cholecystectomy for acute cholecystitis using Gram stain results from a smear preparation of bile.

Materials and Methods

The study protocol was approved by the ethics committee at our hospital (Bell Land General Hospital Ethic Board approval number 2017-0003). This was a retrospective study that did not require patient consent. The computerized medical records of our institution were searched to identify patients who underwent emergency cholecystectomy for acute cholecystitis between January 2011 and December 2016. Accordingly, 185 patients histopathologically diagnosed with acute cholecystitis were identified.

Bile was collected aseptically during surgery in 121 of the enrolled patients. The patients in whom bile was not collected were excluded. Cefazolin was used for prophylaxis just before the operation in all patients. After bile was collected, bile was immediately rolled onto the surface of a glass microscope slide in preparation for Gram staining, and then we immediately poured it into an aerobic and anaerobic transportation container and evaluated minimum inhibitory concentration (MIC). Evaluation of MIC was performed by broth dilution method, and the determination of antimicrobial resistance was carried out using Blake Point proposed by the Clinical and Laboratory Standards Institute. Gram stains of the smear were performed using a standard laboratory procedure in our institute, and all smear slides were reviewed at ×100 magnification and 40 oil immersion (×1000) fields per slide by a highly experienced clinician who was blinded to the physical examination findings. The diagnosis and the severity grade of acute cholecystitis were evaluated according to TG13.[1] In these patients, the correspondence of the Gram stain results from bile smear and culture preparations were evaluated. Furthermore, we created a local antibiogram at our institution and analyzed the pattern of antimicrobial resistance according to the Gram stain results.

Statistical analyses were conducted using χ^2 tests for categorical variables. In 2-tailed tests, $P < 0.05$ was considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama Prefecture, Japan), which is a graphical user interface for R (version 2.13.0, The R Foundation for Statistical Computing, Vienna, Austria).[8]

Results

Patient characteristics

Patient characteristics of 121 patients who had cholecystectomy performed for acute cholecystitis and had bile collected intraoperatively are shown in Table 1. Twenty-one patients (17.4%) had biliary drainage for acute cholangitis preoperatively. Forty-five patients (37.2%) had gangrenous cholecystitis in the histopathologic findings. According to the severity grade of acute cholecystitis, 68 patients (56.2%) were diagnosed grade I, and 53 patients (43.8%) were grade II. The median from onset of symptoms until operation was 2 days, and the number of patients with <3 days from onset of symptoms until operation was 81 patients (66.9%). We encountered postoperative complications in 13

Table 1 Patient characteristics of 121 patients

Variable	Data
Sex, male/female	75 (62.0%)/48 (38.0%)
Age, yr	70 (62–78)
Body mass index, kg/m^2	24.1 (22.0–26.7)
Presence of diabetes mellitus	24 (19.8%)
Presence of hypertension	54 (44.6%)
Presence of liver cirrhosis	1 (0.8%)
Use of antithrombotic agent	37 (30.6%)
Preoperative biliary drainage	21 (17.4%)
Operative procedure, open/laparoscopic	44 (36.4%)/77 (63.6%)
Gangrenous cholecystitis	45 (37.2%)
TG13 classification, grade I/grade II	68 (56.2%)/53 (43.8%)
Days from onset of symptoms until operation, days	2 (1–3)
<3	81 (66.9%)
≥3	40 (33.1%)
Operation-related postoperative complication	13 (10.7%)
Abdominal abscess	7 (5.8%)
Bile leakage	1 (0.8%)
Wound infection	3 (2.5%)
Cholangitis	2 (1.7%)
Hematoma	2 (1.7%)

Data are expressed as median with interquartile range for continuous data or as number and percentage for categorical data. A repetition case was included in these numbers. TG13, 2013 Tokyo Guidelines.

patients, which were abdominal abscess, bile leakage, wound infection, cholangitis, and hematoma.

Gram stain results from the smear and culture preparations

Bile bacteria were detected in 82 patients (67.8%) with the culture preparation and in 72 patients (59.5%) with the smear preparation. In the Gram stain results from the culture preparations, bile bacteria were detected in 48 (59.3%) and 34 patients (85.0%) of the patients who had surgery <3 and ≥3 days from onset of symptoms, respectively ($P = 0.004$). In the Gram stain results from the smear preparations, bile bacteria were detected in 41 (50.6%) and 31 patients (77.5%) who had surgery <3 and ≥3 days from onset of symptoms, respectively ($P = 0.006$). For patients with a TG13 severity of grade I, bile bacteria were detected in 41 of 68 patients (60.3%) with the culture preparation and in 34 of 68 of patients (50.0%) with the smear preparation. For patients with a TG13 severity of grade II, bile bacteria were detected in 41 of 53 patients (77.4%) with the culture preparation and in 38 of 53 of patients (71.7%) with the smear preparation. The average rate of correspondence in the Gram stain results between smear and culture preparations was 86.3% among patients with bile bacteria in the smear preparation. Furthermore, the average rate of correspondence in the Gram stain results was 83.3% for patients with a severity of grade I and was 89.0% for patients with a severity of grade II.

Bacteriological profile according to the TG13 severity grade and Gram stain results

The results of the bacteriologic analysis, stratified by the TG13 severity grade, are shown in Table 2. In the severity grade I group, Gram-positive cocci (GPCs) were isolated from 20 cases (28.6%), Gram-positive rods (GPRs) were isolated from 5 cases (7.1%), and Gram-negative rods (GNRs) were isolated from 45 cases (64.3%). In the severity grade II group, GPCs were isolated from 20 cases (30.0%), GPRs were isolated from 7 cases (9.1%), and GNRs were isolated from 50 cases (64.9%; including a repetition case). There were no significant differences in the isolated bacteria profile according to the Gram stain results between severity grade I and grade II groups. With regard to resistance rate, *Streptococcus anginosus* (25.0%), *Streptococcus sanguinis* (0.0%), *Streptococcus salivarius* (0.0%), *Edwardsiella tarda* (25.0%), and *Prevotella* spp. (0.0%) had low rates of antimicrobial resistance. However, several microorganisms showed high rates of antimicrobial resistance, including *Enterococcus* spp. (100.0%), *Clostridium perfringens* (90.0%), *Escherichia coli* (50.0%), *Bacteroides fragilis* (85.7%), and *Citrobacter freundii* (100.0%).

The antimicrobial resistance rates for each antimicrobial agent according to the Gram stain results are shown in Table 3. Cefepime and meropenem had low antimicrobial resistance for all Gram stain results (<10%). Piperacillin, cefuroxime, cefotaxime, ceftriaxone, cefpirome, cefepime, meropenem, panipenem/betamipron, and daptomycin had no resistance for GPCs, whereas piperacillin, cefpirome, and panipenem/betamipron had no resistance for GPRs. However, among these highly susceptive antimicrobial agents for Gram-positive bacteria, piperacillin (21.3%), cefuroxime (51.2%), cefotaxime (13.4%), cefepime (8.5%), and panipenem/betamipron (23.1%) showed antimicrobial resistance for GNRs. In contrast, meropenem and amikacin had no resistance for GNRs, whereas piperacillin/tazobactam (1.2%), latamoxef (2.6%), imipenem/cilastatin (3.7%), and gentamicin

Table 2 Bacteriologic profiles according to the TG13 severity grade and the antimicrobial resistance rate

Bile bacteria	Grade I (n = 70)	Grade II (n = 75)	*P*	Total antimicrobial resistance rate (%)
Gram-positive bacteria	25	27	0.225	
Enterococcus spp.	8	11		
Enterococcus faecalis	(7)	(4)		100
Enterococcus faecium	(1)	(3)		100
Enterococcus casseliflavus	(0)	(2)		100
Enterococcus avium	(0)	(1)		100
Enterococcus gallinarum	(0)	(1)		100
Streptococcus spp.	12	6		
Streptococcus anginosus	(6)	(2)		25
Streptococcus sanguinis	(2)	(2)		0
Streptococcus salivarius	(2)	(0)		0
Streptococcus constellatus	(1)	(1)		50
Streptococcus bovis II	(0)	(1)		100
Streptococcus intermedius	(1)	(0)		100
Staphylococcus spp.	0	2		
Staphylococcus aureus	(0)	(2)		100
MRSA	(0)	(1)		100
Lactococcus spp.	0	1		
Lactococcus garvieae	(0)	(1)		ND
Clostridium spp.	4	7		
Clostridium perfringens	(3)	(7)		90
Clostridium innocuum	(1)	(0)		100
Bacillus spp.	1	0		100
Gram-negative bacteria	45	50	0.558	
Escherichia coli	10	12		50
Harboring ESBL	(2)	(4)		100
Klebsiella spp.	13	21		
Klebsiella pneumoniae	(7)	(12)		100
Harboring ESBL	(1)	(0)		100
Klebsiella oxytoca	(6)	(9)		100
Bacteroides fragilis	3	4		85.7
Enterobacter spp.	5	3		
Enterobacter cloacae	(4)	(2)		100
Harboring AmpC β lactamase	(1)	(0)		100
Enterobacter aerogenes	(1)	(1)		100
Citrobacter spp.	4	4		
Citrobacter freundii	(3)	(3)		100
Citrobacter braakii	(1)	(0)		100
Citrobacter werkmanii	(0)	(1)		100
Edwardsiella tarda	1	3		25
Aeromonas spp.	3	2		
Aeromonas sobria	(2)	(1)		100
Aeromonas hydrophila	(1)	(0)		100
Aeromonas caviae	(0)	(1)		100
Serratia marcescens	1	0		100
Prevotella spp.	2	0		
Prevotella corporis	(1)	(0)		0
Prevotella oralis	(1)	(0)		0
Anaerobes	3	1		100

A repetition case was included in these numbers. ESBL, extended-spectrum β lactamase; MRSA, methicillin-resistant *Staphylococcus aureus*.

(1.2%) had a low resistance for GNRs. In addition, amikacin (90.0%), imipenem/cilastatin (50.0%), and gentamicin (95.0%) had antimicrobial resistance for GPCs.

Discussion

We found that (1) the rate of correspondence between the Gram stain results obtained with

Table 3 Local antibiogram at our institution according to the Gram stain results and TG13 severity grade

	Antimicrobial resistance rate (%)								
	Gram-positive cocci			Gram-positive rods			Gram-negative rods		
Antimicrobial agents	Total	G I	G II	Total	G I	G II	Total	G I	G II
Benzylpenicillin	16.7	5.6	21.1	ND	ND	ND	ND	ND	ND
Ampicillin	10.8	0	22.2	100	100	ND	81.7	67.6	80
Piperacillin	0	0	0	0	0	0	21.3	19	22.9
Ampicillin/sulbactam	50	ND	50	ND	ND	ND	42.7	54.1	35.6
Piperacillin/tazobactam	ND	ND	ND	ND	ND	ND	1.2	2.7	0
Cefazolin	95.2	100	92.3	ND	ND	ND	43.9	56.8	35.6
Cefotiam	92.9	100	100	ND	ND	ND	22.7	29.4	20.7
Cefuroxime	0	0	0	ND	ND	ND	51.2	45	56.3
Cefdinir	ND	ND	ND	ND	ND	ND	30.6	35	33.3
Cefditoren-pivoxil	ND	ND	ND	ND	ND	ND	17.1	21.4	10.7
Cefpodoxime-proxetil	ND	ND	ND	ND	ND	ND	16.9	23.5	14
Cefmetazole	ND	ND	ND	ND	ND	ND	14.6	18.9	13.3
Cefotaxime	0	0	0	ND	ND	ND	13.4	16.2	11.1
Ceftriaxone	0	0	0	ND	ND	ND	ND	ND	ND
Ceftazidime	ND	ND	ND	ND	ND	ND	13.4	16.2	11.1
Cefpirome	0	0	0	0	0	0	53.8	37.5	80
Cefepime	0	0	0	ND	ND	ND	8.5	8.3	8.9
Latamoxef	ND	ND	ND	ND	ND	ND	2.6	2.9	0
Flomoxef	100	ND	100	ND	ND	ND	ND	ND	ND
Aztreonam	ND	ND	ND	ND	ND	ND	9.8	10.8	8.9
Imipenem/cilastatin	50	ND	50	ND	ND	ND	3.7	0	6.7
Meropenem	0	0	0	ND	ND	ND	0	0	0
Panipenem/betamipron	0	0	0	0	0	0	23.1	12.5	40
Amikacin	90	100	83.3	ND	ND	ND	0	0	0
Gentamicin	95	100	91.7	ND	ND	ND	1.2	0	2.2
Arbekacin	85.7	100	76.9	ND	ND	ND	ND	ND	ND
Erythromycin	45.9	33.3	57.9	100	100	ND	ND	ND	ND
Clindamycin	51.4	45	60	81.8	100	71.4	53.8	75	60
Minocycline	38.1	37.5	38.5	100	100	ND	13	8.8	16.3
Tetracycline	25	10	42.9	ND	ND	ND	ND	ND	ND
Vancomycin	8.1	0	15.8	ND	ND	ND	ND	ND	ND
Teicoplanin	5	0	0	ND	ND	ND	ND	ND	ND
Daptomycin	0	0	0	ND	ND	ND	ND	ND	ND
Levofloxacin	15.2	5.6	26.7	ND	ND	ND	9.8	8.1	11.1
Tosufloxacin	100	100	100	50	40	57.1	61.5	50	80
Ciprofloxacin	ND	ND	ND	ND	ND	ND	12.5	19	6.7
Sulfamethoxazole-trimethoprim	90	100	83.3	ND	ND	ND	7.3	5.4	8.9
Linezolid	5	0	8.3	ND	ND	ND	ND	ND	ND

Abbreviations: G I, severity grade I; G II, severity grade II; ND, not detected.

culture and smear preparations was high, especially in TG13 severity grade II cases; and (2) antimicrobial resistance differed according to the Gram stain results based on the generated antibiogram. From these results, Gram stain results from a smear preparation of bile may be helpful in determining the appropriate perioperative antimicrobial agents to prevent SSIs.

First, this study showed that the smear Gram staining is useful in predicting bile bacteria in acute cholecystitis, especially in TG13 severity grade II cholecystitis. The Gram stain examination is a rapid diagnostic test for infectious disease and can be performed immediately without expensive medical equipment. Furthermore, a Gram stain test with the smear preparation provides immediate results, suggesting that it can be clinically useful in selecting the appropriate perioperative antimicrobial agents. However, Gram stain results with the smear preparation did not completely correspond to those with the culture preparation. This may be because of the detection limits of the Gram stain test because its bacterial detection sensitivity is reported to be 10^4 to 10^5 cfu/mL.[9,10] Moreover, the bacterial detection

sensitivity decreases after using antimicrobial agents.[11] In the present study, all patients were administered antimicrobial agents preoperatively; therefore, the bacterial concentrations may have decreased to a level at which the bacteria could not be detected in the smear preparation. However, this indicates that preoperative antimicrobial agents were effective. Thus, Gram stain results with the smear preparation are useful in predicting the bile bacteria and selecting the appropriate perioperative antimicrobial agents. Furthermore, Gram stain results may also help in evaluating the effect of the antimicrobial agents used preoperatively.

In addition, the present study demonstrated that antimicrobial resistance differs according to the Gram stain results. This result also suggests that the optimal perioperative antimicrobial agents can be chosen according to the Gram stain results. Various antimicrobial-resistant pathogens, especially enterobacteria such as extended-spectrum β lactamase-producing *Escherichia coli* and *Klebsiella* spp., have been shown to be increased in biliary tract infections.[6] Therefore, perioperative antimicrobial agents for acute cholecystitis should be used in accordance with the antibiogram in each institution, local area, and country.[12] The guidelines of the Surgical Infection Society of North America and the Infectious Disease Society of America 2010 (SIS-NA/IDSA 2010 guidelines) recommend carbapenems, piperacillin/tazobactam, and amikacin for empiric antimicrobial therapy if more than 10% to 20% of the community isolates of *Escherichia coli* are resistant.[13] In the present study, the antimicrobial resistance rate of *Escherichia coli* was 50%, and meropenem, amikacin, and piperacillin/tazobactam had low resistance for GNRs at 0%, 0%, and 1.2%, respectively. These results suggest that the SIS-NA/IDSA 2010 guidelines are also appropriate for our institution. However, the amikacin resistance rate for GPCs was 90% in our institution. Therefore, amikacin should not be used in cases where GPCs are detected in the Gram stain results with the smear preparation.

In the present study, there was no significant difference in the isolated bacterial profile between TG13 severity grades. The TG13-recommended duration of antimicrobial therapy for severity grade I acute cholecystitis is 24 hours after the cholecystectomy.[12] In contrast, the recommended duration of antimicrobial therapy for severity grade II acute cholecystitis is 4 to 7 days once the source of the infection is controlled.[12] Effective antimicrobial agents are required as an empiric therapy for preventing SSIs, especially in cases with severity grade II acute cholecystitis. However, an expansion in the antimicrobial resistance is of concern when broad-spectrum antimicrobial agents are freely used. Therefore, in the present study, we investigated whether the Gram stain results of bile can be potentially used for selecting effective narrower-spectrum antimicrobial agents for acute cholecystitis. In the antibiogram at our institution, piperacillin, cefuroxime, cefotaxime, ceftriaxone, cefpirome, cefepime, meropenem, panipenem/betamipron, and daptomycin showed high susceptibility for GPC; piperacillin, cefpirome, and panipenem/betamipron showed high susceptibility for GPR; and piperacillin/tazobactam, latamoxef, meropenem, and amikacin showed high susceptibility for GNR. Carbapenems such as meropenem or panipenem/betamipron, which are broad-spectrum antimicrobial agents, are effective against all bile bacteria. However, if carbapenems are used frequently, carbapenem-based resistance can occur. Therefore, the antimicrobial agents should be chosen according to the Gram stain results. For example, piperacillin should be used if only Gram-positive bacteria are detected in the smear preparation. Accordingly, the incidence of broad-spectrum antimicrobial resistance can be reduced.

The study had several limitations. First, the generated antibiogram was based on the results from only 1 institution. There may be regional differences in bacterial species or drug resistance. Therefore, it is important to continue generating antibiograms at each institution. Second, there are bacteria with low dyeability in the Gram stain examination. Bacteria not detected in the Gram stain examination require confirmation from the bile culture results.

Conclusions

In the present study, the rate of correspondence between the Gram stain results obtained from culture and smear preparations was high, especially in TG13 severity grade II cases, indicating that considering Gram stain results from smear preparation could improve the choice of perioperative antimicrobial agents. Institution-specific antibiograms should be created and perioperative antimicrobial agents should be chosen based on this antibiogram because there may be regional differences.

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