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Title: Quick detection of causative bacteria in cases of acute cholangitis and cholecystitis using a multichannel gene autoanalyzer

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ABSTRACT

Purposes

Acute cholangitis and cholecystitis can become severe conditions as a result of inappropriate therapeutic administration and also become increasingly resistant to antimicrobial treatment. Simultaneous detection of the bacterial nucleic acid and antimicrobial resistance gene is covered by insurance in Japan for sepsis. In this study, we evaluate the use of a multichannel gene autoanalyzer (Verigene system) for the quick detection of causative bacteria in cases of acute cholangitis and cholecystitis.

Methods

This study included 108 patients diagnosed with acute cholangitis or cholecystitis between June 2015 and November 2018. Bacterial culture test and Verigene assay were used to evaluate the bile samples.

Results

The most commonly isolated bacteria were *Escherichia coli* (23.1%), which includes six extended-spectrum beta-lactamase (ESBL)-producing *E. coli*. Among patients with positive bile cultures, bacteria were detected in 35.7% of cases via the Verigene system. The detection rates of the Verigene system

significantly increased when the number of bacterial colonies was ≥ 10^6 colony-forming unit (CFU)/mL (58.1%). Cases with a maximum colony quantity of ≥ 10^6 CFU/mL exhibited higher inflammation, suggesting the presence of bacterial infection.

Conclusions

The Verigene system might be a new method for the quick detection of causative bacteria in patients with infectious acute cholangitis and cholecystitis.

Introduction

Acute cholangitis and cholecystitis are acute inflammatory diseases of the gallbladder or bile duct [1, 2]. Antimicrobial therapy plays an important role in managing patients with acute cholangitis and cholecystitis. However, the emergence of antimicrobial resistance (AMR) among clinical isolates has significantly affected the selection of empirical therapy for patients with intra-abdominal infections, including acute cholangitis and cholecystitis [3].

The Tokyo Guidelines 2018 (TG18) were published to provide guidance on the management of acute cholangitis and cholecystitis [1, 2]. The TG18 recommend bile culture to detect causative bacteria and AMR [[3]. However, these tests typically take about 5 days to confirm antimicrobial susceptibility results. Therefore, we commonly select for initial empirical therapy based on the TG18 recommendation. However, the increase in the incidence of AMR of Enterobacteriaceae has recently become a global problem [4]. Reports have indicated that the proportion of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Japan in all community-acquired and healthcare-associated infections were 10.6% and 10.7%, respectively [5]. In Korea, ESBL-producing *E. coli* accounted for 30.4% of *E. coli* cultured from patients with

acute cholangitis who underwent biliary drainage at an university medical center [6]. In medical university hospitals in India, a total of 66% of *E. coli* were ESBL-producing strains [7]. Moreover, in a university hospital in France, a total of 17% of patients were found to be carriers of ESBL-producing Enterobacteriaceae [8]. Therefore, the incidence of resistant bacteria is predicted to further increase globally in the near future, and selecting antimicrobials as empirical therapy will be difficult for such cases.

At the General Assembly of the World Health Organization (WHO) in May 2015, it was reported that the AMR affects all areas of health, including longer illnesses, increased mortality, prolonged hospital stays, loss of protection for patients undergoing operations, and other medical procedures, and that it impacts the society as a whole [9]. Hence, developing reagents that can rapidly and efficiently diagnose infections is needed. In addition, WHO member countries are required to develop a national action plan to deal with AMR. In Japan, a plan was devised in April 2016 to optimize the administration of antimicrobial agents and reduce AMR [10].

In our previous study [11], we performed metagenomic analysis and next-generation DNA sequencing of bile samples from patients who underwent

cholecystectomy for acute cholecystitis and were able to comprehensively determine the causative bacteria. Metagenomic analysis has been reported to be a new method for detecting the etiological agents of an infectious disease [12]. Pathogens can be inferred by directly sequencing millions of DNA/RNA molecules in specimens and matching that sequence in a database [13]. This method enables the identification of potential causative bacteria and AMR genes within 2 days. However, a time course of more than 2 days for severe acute cholangitis is fatal. Therefore, we undertook a study of the use new devices that provide simultaneous detection of bacterial nucleic acid and AMR genes to enable faster identification of the causative bacteria and AMR genes.

One of these devices is the Verigene system (Nanosphere Inc., Northbrook, IL, USA), which was approved by the U.S. Food and Drug Administration in January 2014 as a multichannel gene autoanalyzer using a microarray method for the rapid identification of causative agents and AMR genes [14]. In June 2017, the Verigene system became covered by insurance in Japan for the treatment of bloodstream infection [15]. According to previous studies, using blood samples from patients with blood infection, the Verigene system showed high accuracy in identifying the causative bacteria [16-18]. However, the

evaluation of a multichannel gene autoanalyzer using bile specimens has not been reported to date.

In the present study, we conducted clinical research to investigate the utility of the Verigene system for the detection of causative bacteria in bile samples from patients with acute cholangitis and cholecystitis.

Methods

Patients

In this study, we retrospectively analyzed consecutive patients who experienced acute cholangitis and cholecystitis from June 2015 to November 2018. Inclusion criteria consisted of patients who provided written informed consent, and from whom bile samples could be collected either percutaneously, intraoperatively, or endoscopically. Meanwhile, exclusion criteria were as follows: patients who did not provide written informed consent, those from whom bile samples could not be collected, and patients younger than 20 years. After applying the inclusion and exclusion criteria, 108 patients were enrolled in this study, including 8 patients with acute cholangitis and 100 patients with acute cholecystitis. The acute cholangitis group did not include patients who

had undergone hepaticojejunostomy. We followed the diagnosis and severity gradings of TG18 for acute cholangitis and cholecystitis [19, 20]. Among the patients with acute cholangitis, 3 cases were categorized as grade III severity, 4 cases as grade II, and 1 case as grade I; among those with acute cholecystitis, 4 cases were categorized as grade III, 59 cases as grade II, and 37 cases as grade I.

The study protocol was approved by the Ethics Committee of Toho University Ohashi Medical Center (approval nos.,14-58, H16045, and H17077) and conducted in accordance with the principles of the Declaration of Helsinki.

Bile samples collection

As recommended by TG18, empirical antimicrobial therapy was initiated after the diagnosis of acute cholangitis and cholecystitis [2, 3]. Based on the patient's conditions, the therapeutic strategy was decided on, such as percutaneous drainage, endoscopic drainage, or surgery. The median duration from initiation of antimicrobial therapy to bile sample collection was 15 hours (range, 0–512 hours). With regard to the method of bile sample collection, among the patients with acute cholangitis, 7 have underwent endoscopic and 1 underwent

percutaneous collection, whereas in the acute cholecystitis group, 87 patients underwent intraoperative, 4 patients endoscopic, and 9 patients percutaneous collection. Bile samples were aseptically collected during surgery or via biliary drainage and divided into three anaerobic porters (Kenki porter®). One porter was immediately transferred to the microbiological department for conventional culture and antimicrobial susceptibility tests; the others were immediately frozen at −20°C for assessment using the multichannel gene autoanalyzer. In the retrospective Verigene analysis, we used the thawed bile sample in the Verigene system assay.

According to the number of isolated bacterial species, the cultured samples were classified into two groups: monomicrobial and polymicrobial. Monomicrobial refers to samples in which only one species of bacteria was isolated in the bile culture, whereas polymicrobial refers to samples with multiple species.

Verigene system assays

Among the gram-positive bacteria, we simultaneously detected 12 bacterial species (*Staphylococcus* spp., *Staphylococcus aureus*, *Staphylococcus*

epidermidis, *Staphylococcus lugdunensis*, *Streptococcus* spp., *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus group*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Listeria* spp.) and three AMR genes (*mecA*, *vanA*, and *vanB*). In the gram-negative bacteria, we detected nine bacterial species (*Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus* spp., *Pseudomonas aeruginosa*, and *Serratia marcescens*) and six AMR genes (CTX-M, KPC, NDM, VIM, IMP, and OXA beta-lactamase genes) [16]. In addition, it took less than 2.5 hours to obtain the results. We performed the Verigene assay and analyzed the results based on the usual methods for sepsis [16, 21]. The Verigene Processor Sp was inset to the extraction tray, utility tray, and test cartridge. The dissolved bile (gram-positive, 350 μL; gram-negative, 700 μL) was then transferred to a specimen well located in the extraction tray, and the Verigene processor was initiated. Nucleic acids from the bile were extracted and hybridized to a microarray. After 2 hours, the microarray was transferred to the Verigene reader for analysis. The Verigene processor was opened after automated nucleic acid extraction and hybridization to a glass array, and the array was transferred to the Verigene reader for

automated reporting of analysis and qualitative results. The Verigene assay includes controls for nucleic acid extraction and array hybridization [6, 16].

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed according to NegEN Combo 1T panels (Microscan Walkaway 96SI; Siemens) in accordance with the criteria of the Clinical and Laboratory Standards Institute [22]. A disk-diffusion test detected ESBL using cefotaxime, ceftazidime, and cefpodoxime with or without clavulanate.

Statistical analysis

Statistical analysis was conducted using the Fisher's exact test for categorical data and the Mann–Whitney *U* test and Kruskal–Wallis test for continuous data. Results were expressed as mean ± standard deviation. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using EZR for Windows, version 14.1 [23].

Results

We enrolled 108 patients in the present study; microorganisms could be cultured in 56 cases (51.9%). **Table 1** shows patient characteristics in the bile culture-positive and culture-negative groups. There was a significant difference between groups in age, American Society of Anesthesiologists physical status (ASA-PS), age-adjusted Charlson Comorbidity Index (CCI), body temperature (BT), and total bilirubin. We performed blood culture testing in 19 cases, with bacteremia diagnosed in seven cases. No mortality was recorded in this study. In addition, among those underwent surgery for acute cholecystitis, no postoperative complication was observed.

Table 2 presents the details of the bacterial species isolated in this study. Of these 56 samples, polymicrobial species were cultured from 23 samples. Finally, a total of 104 strains were isolated. The number of identified bacterial species among the polymicrobial species was as follows: 10 samples with 2 species, 10 samples with 3 species, and 3 samples with more than 4 species. Twenty-seven strains, which were not able to be identified by Verigene, mainly included anaerobic microorganisms and *Candida* spp. The other 77 strains included 60 gram-negative (57.8%) and 17 gram-positive (16.3%) strains. The bacterial isolates were mainly Enterobacteriaceae, including *E. coli* (23.1%),

Klebsiella spp. (20.2%), *Enterobacter* spp. (10.6%), *Streptococcus* spp. (8.7%), *Enterococcus* spp. (7.7%), and *Clostridium* spp. (10.6%). In addition, 6 (25%) of the 24 *E. coli* strains were determined to be ESBL-producing *E. coli*. Other AMR such as carbapenem-resistant Enterobacteriaceae, vancomycin resistant *Enterococci*, and methicillin-resistant *Staphylococcus aureus* were not observed in this study.

Table 3 shows the results of the Verigene assay in relation to the results of the bile culture. In the bile culture-positive group, the same bacteria were significantly identified by Verigene assay in 20 (35.7%) of the cases. In the bile culture-negative group, no bacteria were identified by Verigene assay in 52 (100%) of the cases. Among the ESBL-producing *E. coli*, four of the six (66.7%) strains were detected as having the CTX-M gene. In the monomicrobial group, the same bacteria were identified by Verigene assay in 14 (42.4%) of the cases. However, in the polymicrobial group, all bacteria were identified by Verigene assay in six (26.1%) of the cases.

We performed additional quantitative analysis by the Verigene system using 1+ (10^6 colony-forming unit (CFU)/mL) as the boundary, based on the results of the bile culture test in the hospital. **Table 4** shows the comparison of the number of

bacterial colonies in the bile culture based on the Verigene analysis. Monomicrobial was detected in 15 cases (48.4%), with a maximum colony quantity greater than 10^6 CFU/mL group and in 18 cases (72.0%) with a maximum colony quantity lower than 10^6 CFU/mL group. Using the Verigene assay, bile cultures with a maximum colony quantity greater than 10^6 CFU/mL indicated a significantly higher detection rate (58.1%; $p < 0.001$). In addition, with regard to patient characteristics, the presence of bactibilia with a maximum colony quantity of greater than 10^6 CFU/mL was significantly associated with higher BT levels (**Table 5**). In addition, the CRP value tended to be higher in this group; however, no statistically significant difference was noted.

Table 6 shows a comparison of patient backgrounds between the Verigene positive and negative groups. Age and CRP level were determined to be higher in the Verigene positive group, as compared with the Verigene negative group; however, we did not observe a statistically significant difference.

Discussion

In the present study, we evaluated the multichannel gene autoanalyzer Verigene using bile samples from patients with acute cholangitis and

cholecystitis. Our results show that Verigene was able to identify 35.7% of bacteria in the bile culture-positive samples. In particular, the sensitivity was significantly increased when the quantity of bacteria was greater than 10^6 CFU/mL. Furthermore, in patients with a bacterial quantity greater than 10^6 CFU/mL, the BT and CRP levels were more likely to be higher.

The AMR of Enterobacteriaceae has been widely reported to be a causative microorganism of community-acquired intra-abdominal infections [24]. In particular, bacteria that produce ESBL and carbapenemase (i.e., metallo-beta-lactamase and non-metallo-beta-lactamase) have a significant effect on the choice of empirical treatment for patients with intra-abdominal infection, including acute cholangitis and cholecystitis [25]. In a prospective cohort study in 567 patients with acute cholecystitis involving 116 institutions worldwide, researchers showed that 16 of 96 isolated *E. coli* (16.7%) produced ESBL [26]. The TG18, the international practice guidelines for acute cholangitis and cholecystitis, summarizes the antimicrobial agents to be used in patients with community-acquired and healthcare-associated acute cholangitis and cholecystitis [3]. In addition, the antimicrobial agents recommended for community-acquired infections are classified based on the severity grading.

Asai et al. [27] reported significant differences in patient characteristics (age, BT, and CRP) in the bile culture-positive cases. In addition, in the evaluation of comorbidities, we found significant differences in ASA-PS and age-adjusted CCI in the bile culture-positive cases in this study. The TG18 lists the bacteria commonly found in biliary tract infections [3]. The most frequently isolated bacteria are *E. coli*, followed by *Klebsiella* spp. In gram-positive bacteria, *Enterococcus* spp. and *Streptococcus* spp. were frequently isolated. These findings are similar to the results of our study. With regard to the isolation of AMR, ESBL-producing *E. coli* was isolated in 25% of cases in this study. We did not observe any other evidence of antimicrobial resistance.

We showed that the Verigene assay has 35.7% sensitivity and 100% specificity for identifying the causative agent and 66.7% sensitivity and 100% specificity for identifying CTX-M. Previous studies have evaluated the Verigene assay in sepsis [28, 29]. Those studies reported a sensitivity as high as 96.3%–97.4% when using blood samples. The result of the Verigene assay for bile samples was less sensitive than that of blood samples. In addition, our study showed a decrease in sensitivity to 26.1% in polymicrobial cultures as compared with monomicrobial cultures, although we did not observe a

significant difference. In a previous study, Ledeboer et al. [16] reported a reduced detection rate of multiple molecular assays, including the Verigene assay, in polymicrobial cultures. Dodemont et al. [30] reported that false-negative findings in the presence of a polymicrobial are due to the signal interference between multiple capture probes on the array or to low quantity near the limit of detection. In this present study, 23 samples (21.3%) were determined to be polymicrobial, which was considered to one of the reasons for the decrease in sensitivity.

There has been no previous evaluation study of the Verigene assay using bile samples; thus, we were unable to compare our results with those of other studies. However, we speculated that the result might be related to the quantity of bacteria in the bile. Comparison of Verigene assay results with a maximum colony quantity in bile culture showed that the sensitivity was significantly higher (58.1%) in patients with greater than 10^6 CFU/mL in bile culture. Typically, in blood stream infections, blood samples are incubated until the blood cultures are positive before the test [6, 16, 21]. On the other hand, in our study, we expected that the quantity of bacteria in the bile would be lower than in blood samples, because the bile was not preincubated to prevent the growth of

polymicrobial that are naturally present in the bile. Therefore, it is possible that, in our study, the Verigene assay was unable to detect bile samples with low bacterial quantity. According to Nanosphere Inc., the limit of detection of the Verigene assay ranges from 10^5 to 10^7 CFU/mL [31], which is similar to our findings.

As compared to this study, the 58.1% sensitivity in our study is by no means high. Based on the limits of the Verigene assay, a comparison of patient characteristics and colony quantity showed higher inflammation in samples with a maximum colony quantity of greater than 10^6 CFU/mL in bile culture. In our previous study, we reported that higher inflammation factors, including older age (≥ 65 years), elevated BT (≥ 37.5 °C), and high serum CRP levels (≥ 13 mg/dL), were significant risk factors for positive bile cultures [27]. Therefore, our results suggest that a maximum colony quantity of greater than 10^6 CFU/mL in bile culture could indicate infectious acute cholangitis and cholecystitis. Moreover, in such cases, the Verigene assay could enable the quick detection of the causative bacteria, which may be very useful in actual clinical practice. However, the Verigene assay is not adequately sensitive, so even if Verigene is negative for bactibilia, the physician should use clinical judgment to determine

the presence of infectious cholangitis and cholecystitis in patients of older age, with high BT, and with high CRP levels to administer the appropriate antimicrobial agents.

The quick detection of causative bacteria in acute cholangitis and cholecystitis using Verigene is expected to be a breakthrough diagnostic method in the future, and if this diagnostic method is standardized, it is expected to result in great progress in the selection of antimicrobial agent. However, this study has some limitations: (1) This study was conducted retrospectively and the number of cases was small. These factors led to the low sensitivity of the Verigene assay. Thus, future studies are needed to prospectively collect more cases and reevaluate the sensitivity of this assay. (2) The bile samples were frozen and thawed during the test. This differs from the actual clinical practice, in which the bile is immediately used after collection. (3) Currently, there is no panel in the Verigene system to detect anaerobic bacteria. Therefore, we excluded anaerobic bacteria including *Bacteroides* spp. and *Clostridium* spp. from the evaluation. In the future, it is expected that reagent development for anaerobic bacteria will be required.

In conclusion, the multichannel gene autoanalyzer Verigene system is a new

device that might be used to rapidly detect the causative bacteria in patients with infectious acute cholangitis and cholecystitis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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Table Legends

Table 1 Patient characteristics according to the bile culture results

Table 2 List of bacterial species isolated from the bile culture

Table 3 Results of the Verigene assay in relation to the bile culture results

Table 4 Correlation between the maximum colony quantity of the strain isolated in bile culture and results of the Verigene assay

Table 5 Correlation between the maximum colony quantity of the strain isolated in the bile culture and patient characteristics

Table 6 Patient characteristics according to the Verigene results

Table 1: Patient characteristics according to the bile culture results

Factor	Bile culture positive (n=56)	Bile culture negative (n=52)	*p*
Primary disease (Cholecystitis / Cholangitis)	Aug-48	52 / 0	**0.006**
Methods of the bile collection (Intraoperative / Endoscopic / Percutaneous)	43 / 10 / 3	44 / 1 / 7	**0.009**
Sex (M / F)	32 / 24	30 / 22	1.000
Age (years)	74 (31 - 93)	67 (30 - 93)	**0.029**
ASA-PS	2 (1 - 4)	2 (1 - 4)	**0.021**
Charlson comorbidity index	5 (0 - 11)	3 (0 - 11)	**0.003**
Severity of TG18 (Grage I / II / III)	15 / 36 / 5	23 / 27 / 2	0.141
Body Temparture (℃)	38.1 (36.4 - 39.7)	37.6 (36.7 - 38.8)	**< 0.001**
White blood cell (/μl)	11,500 (5,600 - 32,700)	12,500 (4,900 - 26,400)	0.837
C-reactive protein (mg/dL)	12.62 (0.04 - 39.48)	7.62 (0.07 - 34.00)	0.190
T-Bil (mg/dL)	1.7 (0.7 - 8.4)	1.4 (0.5 - 4.6)	**0.040**
Blood culture test (Present / Absent)	15 / 41	4 / 48	**0.011**
Blood culture positive (Positive / Negative)	6 / 9	1 / 3	1.000
Duration antimicrobial treatment before bile collection (h)	12 (0 - 440)	21 (0 - 512)	0.236
Duration from onset to admission (h)	26 (0 - 432)	19 (0 - 268)	0.624
Duration from admission to bile collection (h)	16 (0 - 351)	23 (1 - 528)	0.107
Duration from onset to bile collection (h)	51 (4 - 528)	59 (3 - 528)	0.481

ASA-PS : American society of anesthesiologists - physical status

Table 2: List of bacterial species isolated from the bile culture

Gram negative bacteria (n=60 strains)	
Escherichia coli	24
ESBL producing *E. coli*	(6)
Klebsiella spp.	21
Klebsiella pneumoniae	(16)
Klebsiella oxytoca	(5)
Enterobacter spp.	11
Enterobacter cloacae	(8)
Proteus spp.	1
Citrobacter freundii	3
Gram positive bacteria (n=17 strains)	
Enterococcus spp.	8
Streptococcus spp.	9
Other (n=27 strains)	
Clostridium spp.	11
Clostridium perfringense	(9)
Bacteroides spp.	3
Others	13

Table 3: Results of the Verigene assay in relation to the bile culture results

	Verigene positive (n=20)	Verigene negative (n=88)	*p*
Bile culture positive (n=56)	20	36	**< 0.001**
Bile culture negative (n=52)	0	52	

	Verigene positive (n=20)	Verigene negative (n=36)	*p*
Monomicrobial (n=33)	14	19	0.264
Polymicrobial (n=23)	6	17	

Table 4: Correlation between the maximum colony quantity of the strain isolated in bile culture and results of the Verigene assay

Maximum colony quantity (CFU/mL) [Mono / Polymicrobial]	Verigene positive (n=20) [Mono / Polymicrobial]	Verigene negative (n=36) [Mono / Polymicrobial]	*p*
$\geq 10^6$ (n=31) [15 / 16]	18 [12 / 6]	13 [3 / 10]	**< 0.001**
$< 10^6$ (n=25) [18 / 7]	2 [2 / 0]	23 [16 / 7]	

Table 5: Correlation between the maximum colony quantity of the strain isolated in bile culture and patient characteristics

Factors	Maximum colony quantity (CFU/mL)			
	Bile culture posiive $\geq 10^6$ (n=31)	Bile culture posiive $< 10^6$ (n=25)	Bile culture negative (n=52)	*p*
Sex (M / F)	15 / 16	17 / 8	30 / 22	0.356
Age	69 (31 - 93)	76 (37 - 89)	67 (30 - 93)	0.091
Body temparture (℃)	38.2 (36.4 - 39.7)	37.9 (37.1 - 39.6)	37.6 (36.7 - 38.8)	**< 0.001**
White blood cell (/μl)	11,500 (5,600 - 32,700)	11,700 (6,200 - 25,900)	12,500 (4,900 - 26,400)	0.644
C-reactive protein (mg/dL)	14.34 (0.04 - 28.52)	9.49 (0.05 - 39.48)	7.62 (0.07 - 34.00)	0.352

Table 6: Patient characteristics according to the Verigene results

Factor	Verigene positive (n=20)	Verigene negative (n=88)	*p*
Primary disease (Cholecystitis / Cholangitis)	18 / 2	82 / 6	0.639
Methods of the bile collection (Cholecystectomy / Endoscopic / Percutaneous)	16 / 3 / 1	71 / 8 / 9	0.652
Sex (M / F)	11 / 9	35 / 53	0.224
Age (years)	73 (53 - 89)	70 (30 - 93)	0.207
ASA-PS	2 (1 - 4)	2 (1 - 4)	0.611
Charlson comorbidity index	5 (2 - 10)	4 (0 - 11)	0.271
Severity of TG18 (Grage I / II / III)	6 / 13 / 1	32 / 50 / 6	0.848
Body Temparture (℃)	38.2 (36.4 - 39.7)	37.7 (36.7 - 39.7)	0.079
White blood cell (/μl)	11,700 (5,600 - 23,400)	12,200 (4,900 - 32,700)	0.997
C-reactive protein (mg/dL)	15.61 (0.05 - 26.37)	8.81 (0.04 - 39.48)	0.318
T-Bil (mg/dL)	1.8 (0.7 - 4.6)	1.4 (0.5 - 8.4)	0.692
Isolated bacterial species (Mono / Poly)	14 / 6	19 / 17	0.256
Blood culture test (Present / Absent)	5 / 15	14 / 74	0.340
Blood culture positive (Positive / Negative)	4 / 1	3 / 11	**0.038**
Duration antimicrobial treatment before bile collecti	4 (0 - 144)	19 (0 - 512)	0.104
Duration from onset to admission (h)	35 (0 - 144)	22 (0 - 432)	0.593
Duration from admission to bile collection (h)	7 (3 - 72)	23 (1 - 528)	**0.027**
Duration from onset to bile collection (h)	54 (10 - 152)	53 (3 - 528)	0.462

ASA-PS : American society of anesthesiologists - physical status