Prevalence, Resistance Rates, and Risk Factors of Pathogens in Routine Bile Cultures Obtained during Endoscopic Retrograde Cholangiography

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Keywords

Acute cholangitis · Bile specimen · Endoscopic retrograde cholangiography

Abstract

Introduction and Objective: Acute cholangitis is a life-threatening condition. The early initiation of antibiotic therapy significantly impacts the course of disease. Only few data are available on distribution and resistance profiles of bile pathogens. Here, we report on an analysis of routinely acquired bile specimens and provide an overview of the prevalence, resistance rates, and risk factors for the presence of pathogens in bile. **Methods:** Bile cultures obtained from 388 endoscopic retrograde cholangiographies (ERCs) with corresponding clinical data were analysed in 208 patients. **Results:** The majority (84.8%) of cultures yielded positive for at least 1 organism. Abundance was highest for *Enterococcus faecalis*, *Enterococcus faecium*, and *Escherichia coli*. Multiresistant organisms were present in 14.9%. The initial antibiotic regimen was changed in 44.1%, which increased the length of hospital stay significantly ($***p < 0.001$). Pre-existing papillotomy (EPT) or biliary drainage was associated with higher frequency of bile pathogens

($**p < 0.01$) in a univariate analysis. Multivariate analysis confirmed these results for EPT and revealed significantly more positive results for pathogens, gram-negative bacteria, and fungi in patients with biliary drainage. Significant differences in the prevalence of pathogens were observed between relevant subgroups of ERC indications. The highest susceptibility rates were observed for linezolid and tigecycline in gram-positive bacteria and for meropenem and gentamicin in gram-negative bacteria. **Conclusions:** Our study provides a comprehensive analysis of the distribution, resistance profiles, and risk factors for the detection of bile pathogens. The frequent change in initial antibiotic treatment highlights the importance of routine bile culture and indicates that current schemas of empirical treatment might not cover the contemporary spectrum of pathogens in bile.

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Introduction

Acute cholangitis is a life-threatening condition, usually caused by obstruction of the biliary system or/and ascending pathogens due to an incompetent or resected sphincter of Oddi [1, 2]. The early interventional restoration of the

bile flow, mostly achieved by endoscopic retrograde cholangiography (ERC), and the early antibiotic therapy are the 2 treatment pillars in the management of acute cholangitis [3–7]. These therapeutic approaches are generally accepted and effective in clinical use. Nevertheless, standardized recommendations of antibiotic treatments are widely lacking, and the choice of the antibiotic regimens is often based on traditional centre-specific protocols. Acute cholangitis was defined by the clinical manifestations of Charcot's triad (consisting of the copresence of fever and/or chills, abdominal pain in the right upper quadrant or epigastric, and jaundice) [5]. Due to the low sensitivity of Charcot's triad for the diagnosis of acute cholangitis, new criteria were established by the Tokyo Guidelines [8, 9].

Acute cholangitis reflects a severe life-threatening condition, which can result in sepsis and death. Despite the fact that bile is sterile in general, asymptomatic biliary colonization was observed in patients [2], who did not show any clinical signs of infection. This condition differs from acute cholangitis. Microbiological cultures alone cannot differ between these 2 conditions in the absence of a clinical assessment. However, routine collection of bile cultures may help to detect current resistance rates and could enable or improve an early targeted therapy and may have an impact on the clinical outcome of patients. Furthermore, the knowledge of local resistance rates may enable antibiotic stewardship strategies.

Several studies investigated the distribution of pathogens in bile specimens in the past [6, 7, 10–13]. However, more contemporary studies are needed to characterize the current pathogen profile and the resistance patterns of pathogens in bile in order to optimize the therapeutic use of antibiotics in times of the increasing antibiotic resistance [14, 15]. Due to an overuse and misuse of antibiotics, we face novel resistances [14] and decreased sensitivity to antibiotics over time. Furthermore, not only pathogens in general but also host factors such as the cause for biliary obstruction, immunodeficiency, or prior biliary interventions may reflect aspects that can influence the choice of antibiotic therapy. Unfortunately, only little evidence is available on host factors and their relevance for the choice of antibiotic therapy regimens. Therefore, new studies on advice of optimal use of antibiotics for biliary infections in modern times are warranted.

Here, we provide an overview on the prevalence and resistance rates of pathogens in bile specimens in general and in subgroups of different causes of biliary obstruction. Furthermore, we provide data on differences in patients with prior papillotomy and biliary drainages as important subgroups compared to controls.

Materials and Methods

This cross-sectional study was conducted at the Hospital of the University Munich, a tertiary centre. Patients were included when a bile culture for microbiological assessment was obtained during ERC between October 2016 and March 2019. During this period, bile cultures from 364 ERCs performed in 208 patients were available for analysis. In particular, bile specimens were collected routinely during this time period independently of indication and clinical suspicion of acute cholangitis, and microbiological culturing was performed. Microbiological data from bile specimens collected in clinical practice before October 2016 from patients who were included in this study according to the above-mentioned criteria were analysed if available ($n = 24$). In total, bile specimens of 388 ERCs in 208 patients were analysed in this study. Data of microbiological samples were correlated with corresponding clinical data. The analysis of patient data was achieved in a retrospective fashion.

Microbiological Culturing Techniques and Antibiotic Susceptibility Testing

Samples were transferred to the microbiology department within 2 h of collection and cultured aerobically and anaerobically according to the laboratory standard procedure. Bacterial identification was performed by MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) using the manufacturer's standard protocol. Spectra were matched with the Bruker Taxonomy database version 4.0.0.2. Susceptibility testing was performed with a BD Phoenix automated AST System (Becton-Dickinson Diagnostic Systems, Heidelberg, Germany) equipped with a software for interpretation of AST results according to the EUCAST breakpoint table (document v6.0, 2017–v8.1, 2018).

Endoscopic Retrograde Cholangiography

ERC was performed using either duodenoscopes or orthograde endoscopes. The duodenoscopes used in this trial belong to the 180er series of Olympus (TJF-Q180V; Japan). Selective cannulation of the common bile duct was indicated in all cases. Operator-dependent wire-guided cannulation with sphincterotome (e.g., double lumen sphincterotomes; Boston Scientific, Marlborough, MA, USA) or catheter (Wieser, Germany) were used in native papillae. Pretreated papillae were cannulated with balloon catheter (Olympus, Tokyo, Japan) or standard catheter with or without wire depending on operator's choice. The bile aspirates analysed in this trial were completely obtained before contrast enhancement.

ERC was performed under conscious sedation as a standard procedure using midazolam, propofol, and/or opioids according to the German guidelines [16]. The guidelines of the German Society of Digestive and Metabolic Diseases (DGVS) recommend a prophylactic antibiotic therapy prior to ERC under certain conditions [17].

Ethic

The study was approved by the ethics committee of the Medical University of Munich (18-013). The study is in line with ethical guidelines of the Declaration of Helsinki.

Statistical Analysis

Statistical calculations were performed with the SPSS 25 software package (IBM, Endicott, NY, USA) using Mann-Whitney U

Table 1. Study population. Summary of baseline characteristics, indications for endoscopic retrograde cholangiography (ERC), and relevant host factors like evidence of papillotomy, biliary drainage, or drug-induced immunosuppression

Basis characteristics	N	%
Patients	208	
ERCs	388	
Gender		
Male	131	63
Female	77	37
Indications		
Cholelithiasis	60	15.5
PSC	34	8.8
Pancreatic carcinoma	57	14.7
Cholangiocellular carcinoma	40	10.3
Hepatocellular carcinoma	8	2.1
LiTx anastomotic stricture	55	14.2
LiTx ITBL	33	8.5
Stent occlusion	6	1.5
Secondary sclerosing cholangitis	8	2.1
Others	87	22.4
Papillotomy		
Prior papillotomy	312	80.4
No papillotomy	73	18.8
Unclear	3	0.8
Stents		
Yes	158	40.7
No	201	51.8
Unclear	29	7.5
Drug-induced immunosuppression		
Yes	41	19.7
No	167	80.3

ERC, endoscopic retrograde cholangiography; PSC, primary sclerosing cholangitis; LiTx, liver transplantation; ITBL, ischaemic type biliary lesion.

test, Pearson's χ^2 test, and generalized estimating equations (GEE) with odds ratio, as applicable. Samples were analysed longitudinally. In the GEE analysis, pre-existing papillotomy and biliary drainage were defined with reference = 1. *p* values <0.05 were considered statistically significant. Data are presented as the means $\pm$ standard errors of the mean.

Results

Baseline Characteristics, Distribution of Pathogens, and Choice of Initial Antibiotic Regimens

A total of 37% (*n* = 77) of the patients in this study were female. Major indications for the intervention were cholelithiasis (15.5%; *n* = 60); primary sclerosing cholangitis (PSC) (8.8%; *n* = 34); ERC due to tumour obstruction

Table 2. Distribution of isolated pathogens in bile

Pathogens	N	Positive in (%) all ERCs
Pathogens in bile (at least 1 pathogen)		
Positive	329	84.8
Negative	59	15.2
Bacteria (10 most frequent)		
<i>Enterococcus faecalis</i>	129	33.2
<i>Enterococcus faecium</i>	111	28.6
<i>Escherichia coli</i>	90	23.2
<i>Enterobacter cloacae</i>	46	11.9
<i>Klebsiella pneumoniae</i>	39	10.1
<i>Klebsiella oxytoca</i>	37	9.5
<i>Enterobacter</i> spp.	34	8.8
<i>Streptococcus</i> spp.	26	6.7
<i>Enterococcus</i> spp.	26	6.7
<i>Pseudomonas aeruginosa</i>	23	5.9
<i>Streptococcus anginosus</i>	22	5.7
<i>Citrobacter freundii</i>	22	5.7
Gram-positive (at least 1 pathogen)	282	72.7
Gram-negative (at least 1 pathogen)	207	53.3
Fungi (3 most frequent)		
<i>Candida albicans</i>	77	19.8
<i>Candida glabrata</i>	20	5.2
<i>Candida tropicalis</i>	10	2.6
ERC, endoscopic retrograde cholangiography.		

(34.3%; *n* = 133), with pancreatic carcinoma (PC), cholangiocellular carcinoma (CCC), and hepatocellular carcinoma (HCC) as leading causes for tumour obstructions (27.1%; *n* = 105); or obstructive problems with or without cholangitis after liver transplantation (LiTx) (22.7%; *n* = 88). Of these patients after LiTx, ERC was indicated and performed in 55 patients due to anastomotic strictures and in 33 patients due to ischaemic type biliary lesion. The majority of ERCs were done in patients, who underwent a prior papillotomy (80.4%; *n* = 312). Biliary stents were present in 40.7% (*n* = 158) of all ERCs. 19.7% of the patients were treated with immunosuppressants after organ transplantation (*n* = 41). Baseline characteristics are given in Table 1. The majority of specimens (84.8%; *n* = 329) were positive for at least 1 pathogen. 21.6% (*n* = 84) were positive for 2 pathogens, 17.8% (*n* = 69) for 3 pathogens, and 22.2% (*n* = 86) were positive for 4 or more pathogens. In 23.2% (*n* = 90), only 1 pathogen was evident.

The 10 most frequently found bacteria are listed in Table 2. The following 3 pathogens were detected most often in bile specimens: *Enterococcus faecalis*, *Enterococcus faecium*, and *Escherichia coli*.

Table 3. Distribution of isolated pathogens of 42 available positive blood cultures associated with ERC

Pathogens	N	Positive in (%) of all positive blood cultures
Bacteria (3 most frequent)		
<i>Escherichia coli</i>	17	40.5
<i>Enterococcus</i> spp. (<i>faecalis</i> et <i>faecium</i>)	16	38.1
<i>Klebsiella</i> spp.	10	23.8
Gram-positive (at least 1 pathogen)	17	40.5
Gram-negative (at least 1 pathogen)	31	73.8

ERC, endoscopic retrograde cholangiography.

Table 4. Initial antibiotic regimens in our study population and the frequency of change during hospital stay

Initial antibiotic regimens	N	%
Antibiotic regimen		
Ceftriaxone	48	23.1
Piperacillin-tazobactam	42	20.2
Ciprofloxacin	14	6.7
Meropenem	9	4.3
Piperacillin-tazobactam + linezolid	4	1.9
Piperacillin-tazobactam + ciprofloxacin	2	1
Meropenem + linezolid	3	1.4
Others	48	23.1
Missing documentation	1	0.5
No anti-infective therapy	37	17.8
Change of antibiotic regimen		
No	90	52.9
Yes	75	44.1
Missing documentation	5	2.9

72.7% ($n = 282$) of specimens yielded positive for gram-positive and 53.3% ($n = 207$) for gram-negative bacteria. 24.2% ($n = 94$) of all bile samples were positive for at least 1 fungus, with *Candida albicans* detected most often (19.8%; $n = 77$). 61 multiresistant bacteria were detected in 58 samples (14.9%). 9.3% ($n = 36$) of all cultures yielded positive for extended-spectrum beta-lactamase-producing gram-negative Enterobacterales (ESBL), with 1 carbapenemase-positive *Enterobacter cloacae*; 2.8% were positive for vancomycin-resistant *Enterococcus faecium* ($n = 11$); and 2.3% were positive for linezolid-resistant pathogens ($n = 9$). Carbapenem resistance among all gram-negative was 3.1% of all cultures.

Contrary to bile samples, blood cultures were not collected routinely in this study. In 42 cases (10.8% of all ERCs), blood cultures associated with ERC were collected

Fig. 1. Patients in whom a change of the antibiotic regimen was performed showed a longer hospital stay. The figure illustrates the duration of hospital stay (in days) in patients in whom a change or no change of antibiotic treatment was conducted ($n_{\text{no change}} = 90$ vs. $n_{\text{change}} = 75$; Mann-Whitney U tests; *** $p < 0.001$).

and yielded positive results for pathogens. The most prevalent pathogens in blood cultures are given in Table 3. In 69.05% ($n = 29$) of all positive blood cultures, at least 1 congruent pathogen was identified in bile cultures. Of those congruent blood-bile samples, 59.5% ($n = 25$) had 1 congruent pathogen, 7.1% ($n = 3$) had 2, and 1 sample had 3 (2.4%) different pathogens that were found in both specimens.

In our centre, the most common empirical treatments were ceftriaxone ($n = 48$), piperacillin-tazobactam ($n = 42$), and ciprofloxacin ($n = 14$) (Table 4). 81.7% ($n = 170$) of the patients received an antibiotic therapy either therapeutically or as an antibiotic prophylaxis in the context of biliary intervention. In 44.1% ($n = 75$) of all patients who received an antibiotic therapy at initial ERC, a change of antibiotic regimen was conducted afterwards. Patients in whom the treatment was changed showed a significant longer hospital stay (9 ± 0.7 vs. 15 ± 1.4 days; $n_{\text{no change}} = 90$ vs. $n_{\text{change}} = 75$; Mann-Whitney U tests; *** $p < 0.001$) (Fig. 1).

In 74 cases, a clinical indication for a change of antibiotic regimen was obvious. In 56.8% ($n = 42$) of these patients, the antibiotic therapy was changed as a consequence of the identified resistance profile of bile pathogens, and in 17.6% ($n = 13$), the antibiotic treatment was escalated due to signs of infection or sepsis.

A change of the antibiotic regimen was conducted in 31.8% ($n = 21$) of patients initially treated with ciprofloxacin only. Antibiotic treatment was modified in 37.9% ($n = 11$) of patients who received empirical treatment with ceftriaxone and in 42.1% ($n = 40$) of the patients who received piperacillin-tazobactam up front.

Table 5. Pre-existing papillotomy and biliary drainage as risk factors for detection of pathogens in bile

Variable	Evidence of pathogen (n)	No evidence of pathogen (n)	N	χ^2 (df)	p value
Gram-positive					
No papillotomy	39	34	73	11.57 (1)	<0.01
Pre-existing papillotomy	101	31	132		
Gram-negative					
No papillotomy	18	55	73	16.23 (1)	<0.001
Pre-existing papillotomy	71	61	132		
Fungi					
No papillotomy	6	67	73	10.48 (1)	<0.01
Pre-existing papillotomy	36	96	132		
Gram-positive					
No stents	81	55	136	14.80 (1)	<0.001
Stents	47	6	53		
Gram-negative					
No stents	42	94	136	30.87 (1)	<0.001
Stents	40	13	53		
Fungi					
No stents	21	115	136	14.75 (1)	<0.001
Stents	22	31	53		

Evidence of gram-positive bacteria, gram-negative bacteria, and fungi in patient after EPT or with biliary drainages was compared with patients without pre-existing EPT or without drainages (χ^2 test, ** $p < 0.01$; *** $p < 0.001$).

Microbiological Pathogens Are Found More Often in Patients with Pre-Existing Papillotomy and Biliary Drainage

We next assessed if patients with pre-existing papillotomy (EPT) or biliary drainages show higher rates of positive microbiological results in bile specimens. Here, we found in a univariate analysis that after EPT or pre-existing biliary drainage, microbiological assessments yielded more often positive for at least 1 gram-positive bacterium, at least 1 gram-negative bacterium, or at least 1 fungus (Table 5).

GEEs confirmed a pre-existing EPT as risk factor for the detection of pathogens, gram-positive bacteria, gram-negative bacteria, and fungi (Table 6a–d). Biliary drainages were significantly associated in the multivariate analysis with the detection of pathogens, gram-negative bacteria, and fungi (Table 6a, c, d). For the detection of gram-positive bacteria, we observed a trend ($p = 0.077$) for biliary drainages as risk factor (Table 6b).

Profile of Pathogens in Subgroups

As relevant subgroups of indications for ERC, we defined cholelithiasis ($n = 60$), PSC ($n = 34$), obstructive biliary problems after LiTx (ischaemic type biliary lesion or anastomotic strictures) ($n = 88$), and ERCs due to tu-

mour obstruction, with PC, CCC, and HCC as leading causes for tumour obstructions ($n = 105$). In a next step, we performed a subgroup analysis comparing these major indications for ERC in our centre using GEE.

The analysis of these most common indications for ERCs in our collective revealed significant differences in the prevalence of gram-positive bacteria, gram-negative bacteria, and fungi evident between the analysed subgroups (Table 7). Of note, in patients with PSC, gram-positive bacteria were identified less frequently, gram-negative bacteria were predominately observed in patients after LiTx, and fungi were predominant in patients who received an ERC due to tumour obstructions (Table 7). These groups significantly differed from other subgroups as illustrated in Table 7.

Resistance Profiles of Pathogens

We next analysed the resistance profiles of pathogens in bile. Resistance profiles of isolated bacteria are listed in Table 8 and those of fungi in Table 9. As expected, most gram-positive bacteria were sensitive for tigecycline ($n_{\text{sensitive}} = 256$, $n_{\text{all}} = 263$; 97.3%), linezolid ($n_{\text{sensitive}} = 294$, $n_{\text{all}} = 303$; 97%), and vancomycin ($n_{\text{sensitive}} = 294$, $n_{\text{all}} = 330$; 89.1%). Gram-negative bacteria were susceptible to meropenem ($n_{\text{sensitive}} = 312$, $n_{\text{all}} = 329$; 94.8%), gentamicin

Table 6. Analysis of risk factors for the positive bile cultures

Variable	Regression coefficients	<i>p</i> value	Odds ratio (95% CI)
<i>(a) Risk factor assessment for bile specimens that yielded positive results for pathogens</i>			
Pre-existing papillotomy	1.241	< 0.01	3.365 (1.632–6.941)
Biliary drainage	0.989	< 0.05	1.692 (1.041–6.935)
<i>(b) Risk factor assessment for bile specimens that yielded positive results for gram-positive bacteria</i>			
Pre-existing papillotomy	0.822	< 0.05	2.276 (1.217–4.256)
Biliary drainage	0.526	0.077	1.682 (0.945–3.031)
<i>(c) Risk factor assessment for bile specimens that yielded positive results for gram-negative bacteria</i>			
Pre-existing papillotomy	0.989	< 0.01	2.69 (1.355–5.33)
Biliary drainage	1.258	< 0.001	3.519 (2.033–6.091)
<i>(d) Risk factor assessment for bile specimens that yielded positive results for fungi</i>			
Pre-existing papillotomy	0.985	< 0.05	2.677 (1.125–6.372)
Biliary drainage	0.842	< 0.01	2.321 (1.371–3.932)

By using a generalized estimating equation, we evaluated pre-existing papillotomy and biliary drainages as risk factors for detection of pathogens in bile for (a) pathogens, (b) gram-positive bacteria, (c) gram-negative bacteria, and (d) fungi ($n = 356$; GEE [generalized estimating equations], binomial logit model).

Table 7. Analysis of distribution of pathogens in relevant subgroups

Subgroups	Mean difference	<i>p</i> value
Gram-positive		
Cholelithiasis versus PSC	0.32	< 0.01
PSC versus obstructive problems after liver transplantation	−0.38	< 0.01
PSC versus other indications	−0.24	< 0.05
Tumour obstruction versus obstructive problems after liver transplantation	−0.16	< 0.05
Obstructive problems after liver transplantation versus Other indications	0.14	< 0.05
Gram-negative		
Cholelithiasis versus obstructive problems after liver transplantation	−0.28	< 0.01
Cholelithiasis versus other indications	−0.17	< 0.05
Fungi		
Cholelithiasis versus tumour obstruction	0.16	< 0.05
PSC versus tumour obstruction	0.21	< 0.05
Tumour obstruction versus obstructive problems after liver transplantation	−0.014	0.051

Evidence of gram-positive bacteria, gram-negative bacteria, and fungi was compared between the major subgroups who received an ERC in our centre ($n = 374$, generalized estimating equations; * $p < 0.05$; *** $p < 0.001$). PSC, primary sclerosing cholangitis.

($n_{\text{sensitive}} = 303$, $n_{\text{all}} = 325$; 93.2%), tigecycline ($n_{\text{sensitive}} = 228$, $n_{\text{all}} = 298$; 82.2%), and cefepime ($n_{\text{sensitive}} = 263$, $n_{\text{all}} = 320$; 82.2%) in >80%. The majority of gram-negative bacteria were susceptible also to ciprofloxacin ($n_{\text{sensitive}} = 262$, $n_{\text{all}} = 329$; 79.6%), piperacillin-tazobactam ($n_{\text{sensitive}} = 239$, $n_{\text{all}} = 328$; 72.9%), and cefotaxime/ceftriaxone ($n_{\text{sensitive}} = 236$, $n_{\text{all}} = 329$; 71.7%). In respect of susceptibility to both gram-positive and gram-negative bacteria, only tigecycline ($n_{\text{sensitive}} = 484$, $n_{\text{all}} = 561$; 86.3%)

and piperacillin-tazobactam ($n_{\text{sensitive}} = 490$, $n_{\text{all}} = 681$; 72%) demonstrated an effectiveness with susceptibility rates higher than 70%. Interestingly, the majority of all detected fungi were susceptible to the tested antifungal agents, like fluconazole ($n_{\text{sensitive}} = 85$, $n_{\text{all}} = 112$; 75.9%), flucytosine ($n_{\text{sensitive}} = 104$, $n_{\text{all}} = 111$; 93.7%), voriconazole ($n_{\text{sensitive}} = 85$, $n_{\text{all}} = 86$; 98.8%), echinocandin ($n_{\text{sensitive}} = 104$, $n_{\text{all}} = 111$; 93.7%), or amphotericin B ($n_{\text{sensitive}} = 20$, $n_{\text{all}} = 22$; 90.9%).

Table 8. Resistance profiles of isolated bacteria in bile

Resistance profiles of pathogens (bacteria)	Sensitivity Gram-positive, %	Sensitivity Gram-negative, %	Sensitivity all, %
<i>Antibiotic regimen</i>			
Ampicillin/amoxicillin	68.1	3.4	37.0
Amoxicillin-clavulanic acid	70.4	20.9	46.4
Piperacillin	69.2	42.0	56.0
Piperacillin-tazobactam	71.1	72.9	72.0
Cefuroxime	12.3	45.8	28.4
Cefotaxime/Ceftriaxone	12.8	71.7	41.2
Cefepime	11.9	80.4	45.8
Ciprofloxacin	2.6	79.6	42.5
Meropenem	15.0	94.8	53.4
Gentamicin	3.2	93.2	49.1
Tigecycline	97.3	82.2	86.3
Linezolid	97.0	n/a	n/a
Vancomycin	89.1	n/a	n/a

Discussion/Conclusion

In light of an ever-increasing number of ERCs, current resistance profiles of bile pathogens in times of increasing antibiotic resistance are warranted [14, 15]. Moreover, identification of host factors predisposing to bile colonization as risk factor for the development of biliary infection [18] and the resistance patterns should be taken into account. Aim of this study was to determine the prevalence of pathogens and their resistance profiles and the identification of risk factors for their presence in bile. This information could influence the choice of empirical antibiotic therapy and may have an impact on the course of acute cholangitis.

Despite a definition of acute cholangitis provided by the Tokyo Guidelines [9], we often face the problem to establish the diagnosis of acute cholangitis definitely in clinical practice. Especially in patients with tumour obstruction or in patients after LiTx, who are highly susceptible to multiple infection foci due to immunosuppression, the establishment of the diagnosis of acute cholangitis can be challenging or even not definitely possible. These situations often require a pragmatic approach in the treatment of these patients. Furthermore, a recent study [19] identified an inadequate initial antimicrobial therapy being associated with increased 30-day mortality in patients with bacteraemic cholangitis. In our opinion, this strongly argues for a routine collection of bile culture during ERC.

In this analysis, we provide data on acquired bile specimens independently of signs of cholangitis. In this study,

Table 9. Resistance profiles of isolated fungi in bile

Resistance profiles of pathogens (fungi)	N	Sensitivity, %
<i>Antimycotic regimen</i>		
Fluconazole	112	75.9
Flucytosine	111	93.7
Voriconazole	86	98.8
Echinocandin	111	93.7
Amphotericin B	22	90.9

we included results from bile cultures that were collected during 388 ERCs in 208 patients between October 2016 and March 2019. We have to state that 388 ERCs reflect less than the estimated number of ERCs during a 2.5-year period in our centre. All colleagues who performed ERCs in our centre for this study were introduced to collect bile cultures routinely. Possible explanations for the discrepancy of estimated and included results from ERCs may be because of the fact that in some ERCs, bile cultures cannot be aspirated due to technical reasons before contrast enhancement. This fact clearly reduced the number of bile samples. If an ERC was performed shortly after first ERC, examiner may have not seen the need to collect a second bile culture. The summary of these reasons may explain the lower number of available cultures for analysis and may reflect a potential bias.

Furthermore, we cannot certainly differentiate between acute cholangitis and biliary colonizations. However, by routinely acquiring bile cultures, we can address important

aspects in the treatment of acute cholangitis, a condition which is challenging to diagnose in clinical practice as mentioned above. First, we can provide an overview of distribution of pathogens and resistance profiles, which may enable antibiotic stewardship strategies in case of acute cholangitis. Second, the early analysis of bile cultures can allow or improve an early targeted therapy and may have an impact on clinical outcomes of patients.

Our analysis revealed a spectrum of microorganisms that is comparable to the results of a large multi-centre retrospective study that investigated >6,000 patients diagnosed with acute cholangitis in Japan and Taiwan [13]. In detail, the study by Gomi et al. [13] and our study showed that about 80% (83.4% [13] vs. 84.8% in this study) of all bile cultures yielded positive results. Both studies identified *E. faecalis*, *E. faecium*, *E. coli*, and *Klebsiella pneumoniae* being highly prevalent microorganisms in bile.

The microbiological results of blood cultures obtained in this study identified *E. coli*, *Enterococcus* spp., and *Klebsiella* spp. as the most prevalent pathogens in blood. This may indicate that all of these pathogens are of pathogenic relevance, and the results are in accordance with the distribution of microorganisms found in routinely obtained bile specimens.

In our opinion, this may underline that results from routinely acquired bile culture are comparable to the spectrum of isolates obtained from patient with acute cholangitis. Prospective studies have to address if the outcome of acute cholangitis can be improved by targeting these pathogens.

Our data illustrate that in about 44% of patients, the antibiotic regimen had to be adjusted during hospital stay. We observed that patients show a significant longer hospital stays when an adjustment of antibiotic regimen was conducted. We cannot rule out that this finding is also related to the cause or severity of underlying disease, which may prolong the hospital stay and lead to an adjustment of the antibiotic regimen independently. However, the high number suggests that standard antibiotic regimens are insufficient, and it highlights the need for more efficient regimens in the treatment of biliary infections. The reason for these high rates of adjustments may be related to the high prevalence of *E. faecium* (28.6%) in bile samples, and it raises the question if initial antibiotic regimens should be considered to have activity against *E. faecium*. Of note and in accordance with a previous study by Rupp et al. [6], in our study, more bile culture yielded positive for gram-positive than for gram-negative bacteria. This raises the question if 3rd generation cephalosporins or ciprofloxacin, which are commonly used in therapy of biliary infections,

reflect an adequate choice for monotherapy as neither the study by Rupp et al. [6] nor the present study revealed an adequate susceptibility of gram-positive bacteria to 3rd generation cephalosporins or ciprofloxacin.

We have to state as limitation of this study that only patients who were treated at a tertiary centre were included. At tertiary centres, the rate of patients, who have already had a prior ERC, is high and the evaluated pathogens may not mirror profiles of all patients who undergo ERC. However, an effective initial antibiotic treatment is of paramount importance for patients in these centres, as they are often more advanced ill and less tolerant to a delay in effective treatment.

In this study, we provide resistance profiles of a broad spectrum of antibiotic substances. We found that in gram-positive bacteria, tigecycline, linezolid, and vancomycin demonstrated high susceptibility rates. In gram-negative bacteria, ciprofloxacin, piperacillin-tazobactam, and 3rd generation cephalosporins (cefotaxime/ceftriaxone) showed acceptable resistance rates. For meropenem, gentamicin, tigecycline, and cefepime, susceptibility rates were above 80%. In our opinion, along with tigecycline, only piperacillin-tazobactam demonstrated acceptable effectiveness in both gram-positive and gram-negative bacteria, indicating a potential agent for an antibiotic initial regimen. It seems contradictory to this assumption when we describe in this study that 42.1% of patients, in whom a change of the antibiotic regimen was conducted, received up front piperacillin-tazobactam. One might argue that patients in whom an initial treatment with piperacillin-tazobactam was selected were more severely ill, which might have an independent influence on the adjustment of antibiotic regimens over course. We have to state here the limitation of the retrospective nature of this study, where changes or adjustments of anti-microbiological treatments were not recorded in a standardized fashion and might cause a bias in this regard.

In general, the high rates of positive microbiological results for pathogens (84.8 with 72.7% positive for gram-positive pathogens and 53.3% positive for gram-negative pathogens) in bile and the fact that only tigecycline or piperacillin-tazobactam seems to be acceptable monoregimes raises the question if combined antibiotic treatments that cover both the gram-positive and gram-negative spectrum should be recommended for the treatment of biliary infections.

Fungal drug resistance is a challenging topic, as the number of antifungal agents for clinical use is low and infections with more resistant *Candida* species are on the rise [20]. In our study, susceptibility rates for *Candida*

spp. were higher for echinocandins than for fluconazole, but further data on the prevalence of *Candida* species in bile samples are needed to address if echinocandins should be preferred in the treatment of critically ill patients with biliary infections.

We demonstrated in this study that pre-existing EPT is a risk factor for positive microbiological testing in bile specimens. Due to the retrospective nature of our study, we cannot certainly define which patients received their “first in life” ERC. However, when we assume that patients in whom an EPT was performed during in-house intervention had their first ERC, we found *E. faecium* in 16.4% ($n = 12$), which may be lower compared to the whole collective, which shows positive testing for *E. faecium* in 28.6%. The high rate of pre-existing EPTs in our centre may be related to frequent indications for recurrent ERCs, especially seen in patients with tumour obstruction or after LiTx.

The subgroup analyses of the most common indications for ERCs in our collective revealed that there are significant differences in frequency of gram-positive bacteria, gram-negative bacteria, and fungi evident between the analysed subgroups (Table 7). This is an important finding as these host factors may influence the choice of treatment. In our opinion, further prospective studies should validate our finding that patients after LiTx show more frequently positive results for gram-negative bacteria and patients with tumour obstructions due to PC, CCC, and HCC are more often positive for fungi. Both subgroups are in general patients where an initial treatment failure may cause more severe clinical courses as these patients have generally more advanced illness. If these findings can be validated in prospective studies, protocols may imply the use of effective therapies for gram-negative bacteria or fungi in these subgroups.

Inpatient care is an important generator of healthcare costs as demonstrated in the elderly in Germany [21]. We assume that the current empirical treatment in our centre is insufficient in regard to its cost-effectiveness caused by the high rates of changes/adjustments of antibiotic therapy (44.1% in this study). A change/adjustment of antibiotic therapy was associated with a significant longer hospital stay (9 ± 0.7 vs. 15 ± 1.4), which clearly indicates relevance for cost-effectiveness. We argue that routine analyses of resistant rates and taking these rates into account for antibiotic therapy may result in shorter hospital stays and may enable a more cost-effective treatment for patients with biliary infections.

We take the view that (a) bile culture should be routinely collected in each ERC independently of acute chol-

angitis to enable early and targeted treatment and (b) further studies should also imply next-generation sequencing that shows high sensitivity for detection of microbiological pathogens [22]. In summary, our study provides a comprehensive analysis of the distribution and resistance profile in patients treated in a tertiary centre. Further prospective studies should be conducted to validate our findings to provide optimized treatment protocols for patients with acute cholangitis.

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Statement of Ethics

The published research complies with the guidelines for human studies and are ethically in accordance with the World Medical Association Declaration of Helsinki. The study was approved by the ethics committee of the Medical University of Munich (18-013).

Conflict of Interest Statement

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Author Contributions

F.P.R., W.O., and C.S. act as the submission's guarantors. F.P.R., W.O., and C.S. collected the data. F.P.R., W.O., U.M.M., and C.S. analysed the data. F.P.R., W.O., and C.S. designed the study and wrote the paper. J.J., G.D., U.M.M., E.N.T., J.S., and J.M. contributed to the design of the study and interpreted the data. All authors approved the final version of the article, including the author list.

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