RESEARCH ARTICLE

Risk Factors for Multi-Drug Resistant Pathogens and Failure of Empiric First-Line Therapy in Acute Cholangitis

Philipp A. Reuken1*, Dorian Torres1, Michael Baier2, Bettina Löffler2, Christoph Lübbert3, Norman Lippmann4, Andreas Stallmach1,5, Tony Bruns1,5

1 Department of Internal Medicine IV (Gastroenterology, Hepatology, and Infectious Diseases), Jena University Hospital, Jena, Germany, 2 Division of Infectious Diseases and Tropical Medicine, Department of Gastroenterology and Rheumatology, Leipzig University Hospital, Leipzig, Germany, 3 Institute of Medical Microbiology, Jena University Hospital, Jena, Germany, 4 Institute for Medical Microbiology and Epidemiology of Infectious Diseases, Leipzig University Hospital, Leipzig, Germany, 5 The Integrated Research and Treatment Center for Sepsis Control and Care (CSCC), Jena University Hospital, Jena, Germany

* philipp.reuken@med.uni-jena.de

Abstract

Background

Acute cholangitis (AC) requires the immediate initiation of antibiotic therapy in addition to treatment for biliary obstruction. Against a background of an increasing prevalence of multi-drug resistant (MDR) bacteria, the risk factors for the failure of empiric therapy must be defined.

Methods

Using a pathogen-based approach, 1764 isolates from positive bile duct cultures were retrospectively analyzed to characterize the respective pathogen spectra in two German tertiary centers. Using a patient-based approach, the clinical and laboratory data for 83 patients with AC were assessed to identify risk factors for AC with pathogens resistant to the applied empiric therapy.

Results

Bile cultures were predominantly polymicrobial, and empiric antibiotic therapies did not cover the full biliary pathogen spectrum in 78% of cases. MDR bacteria were isolated from the bile of 24/83 (29%) patients. The univariate risk factors for biliary MDR bacteria were male sex, nosocomial AC, prior antibiotic exposure and prior biliary stenting, of which biliary stenting was the only independent risk factor according to multivariate analysis (OR = 3.8; 95% CI 1.3–11.0, \( P = 0.013 \)). Although there were no significant differences in survival or hospital stay in AC patients with and without detected biliary MDR pathogens, the former more often had a concomitant bloodstream infection (58% vs. 24%; \( P = 0.019 \)), including those involving MDR pathogens or fungi (21% vs. 2%; \( P = 0.007 \)).
Conclusion

Patients with biliary stents who develop AC should receive empiric therapy covering enterococci and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae. These patients are at an increased risk for bloodstream infections by MDR pathogens or fungi.

Introduction

Acute cholangitis (AC) is a potentially life-threatening bacterial infection of the intra- and/or extrahepatic bile ducts (BD) caused by obstruction of the BD, with stasis and subsequent infection of the bile [1–3]. Common causes of AC are gallstones, BD stenosis in cases of chronic pancreatitis, malignomas and sclerosing cholangitis [1]. The typical symptoms are fever, jaundice and abdominal pain (Charcot’s triad) [4]. Current treatment strategies support a risk-stratified approach based on the revised Tokyo Guidelines [1,5] and generally comprise a combination of antibiotic therapy and early endoscopic resolution of the obstruction [6] because delaying endoscopic treatment often results in persistent organ failure [7].

The pathogens most frequently isolated from bile are Gram-negative Enterobacteriaceae, such as Escherichia coli and Klebsiella spp., as well as Enterococcus spp., with a high proportion of polymicrobial cultures observed [8,9]. Enterococcus spp. were the biliary pathogens most frequently observed in a recent study from a German tertiary center, with an even higher proportion of Enterococcus spp. found in patients who had undergone BD stenting [10]. Bile from healthy individuals is sterile; however, in patients with BD pathologies, bacterial or fungal colonization of the bile without clinical signs of infection may occur [11,12] in up to 100% of patients with biliary stents [13], which can be difficult to discriminate from AC [14].

Because the microbiological identification of pathogens requires time, antibiotic therapy is generally initiated as an empiric therapy. Current guidelines recommend treatment with third-generation cephalosporins (3GC) or a penicillin/beta-lactamase inhibitor-based agent as first-line options for empiric therapy [15,16], initiated as an intravenous infusion. An early switch to oral therapy was found to be non-inferior compared with continuing intravenous infusion [17].

Because microbes and resistance patterns show both regional and temporal variations [18,11,19], the aims of this retrospective study were to (i) characterize the contemporary microbial patterns of BD cultures performed in two German tertiary care centers using a pathogen-based approach and (ii) identify risk factors for AC by pathogens resistant to empiric antibiotic therapies and the associated outcomes using a patient-based approach.

Methods

Study design

To characterize the biliary pathogen spectrum and resistance patterns, microbiological data were reviewed to identify all patients with positive bile duct cultures performed at the Jena University Hospital (JUH) between 1996 and 2012 and at the Leipzig University Hospital (LUH) between 2013 and 2015. Cases in which identical pathogen spectra were identified in individual patients during the same hospital stay (copy strains) were excluded from the analysis.
A subset of patients (N = 83) who were admitted between 2006 and 2012 to the JUH were used to identify individual risk factors for acquiring resistant organisms. For this retrospective approach, medical records, including patient files, electronic health records, imaging data, laboratory data, and nursing documentation, were used to retrospectively collect clinical and laboratory data. The following variables were collected from the medical records: age; gender; comorbidities; medications, including antibiotics and changes in antibiotic therapy; treatment in the intensive care unit (ICU); endoscopic diagnostic procedures and interventions; length of hospital stay and mortality. Nosocomial AC was defined in patients who were hospitalized for 48 h or more before bile fluid sampling because the onset of their symptoms was less well defined. Immunosuppressive therapy was defined as treatment with high-dose prednisolone, azathioprine, anti-TNF-alpha antibodies, calcineurin-inhibitors, such as cyclosporine A or tacrolimus, or cytostatic chemotherapy. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the internal review board (local ethics committee; registry number 4734-03/16).

Microbiological sampling

Samples for blood cultures (BC) were collected before or immediately after the initiation of antibiotic treatment by the bedside inoculation of 10 ml of blood into BC bottles (BacT/Alert, bioMérieux, Durham, NC, USA). Bottles were incubated at 37˚C until microbial growth was detected or for at least seven days. Bile cultures were obtained via endoscopic retrograde cholangiopancreatography (ERCP) using catheter aspiration. Primary of bile cultures, as well as all subcultures, including those of positive BC cultures, were performed using standard solid media, e.g., Columbia blood agar (SIFIN, Berlin, Germany) for aerobic bacteria and Schaedler agar (Oxoid, Basingstoke, UK) for anaerobic bacteria. The cultivated microorganisms were identified and antibiotic susceptibility testing (MIC) was performed using a VITEK 2 system (bioMérieux, Durham, NC, USA), with the results interpreted according to the DIN- and EUCAST guidelines [20].

Statistical analysis

Statistical analyses were performed using SPSS 20 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY:IBM Corp) and Prism 5 (GraphPad Software, La Jolla, CA, USA) software. Significant differences were assessed using the nonparametric Mann-Whitney U test or the Kruskal-Wallis test for continuous data or Fisher’s exact test for discrete variables, as appropriate. The risk factors for cholangitis with resistant pathogens were determined by multivariate binary logistic regression, including using significant univariate predictors through stepwise backward elimination. The significance level in two-sided testing was P < 0.05, without correction for multiple testing.

Results

Overall pathogen spectra and resistance profiles at two tertiary centers

Overall, 1764 isolates were identified from 531 bile cultures (1504 pathogens from 419 cultures at the JUH and 260 pathogens from 112 cultures at the LUH). Seventy-eight percent of cultures were polymicrobial, with a median of two isolated microorganisms (bacteria or fungi) per culture (range: 1–9). On the family level, the most frequently isolated pathogens were Enterobacteriaceae (715 isolates; 40.5% of all identified pathogens) and Enterococcaceae (440 isolates; 24.9% of all identified pathogens). On the species level, Escherichia coli was the most frequently detected pathogen (282 isolates; 16% of all identified pathogens), followed by Enterococcus faecalis (236 isolates; 13.4% of all identified pathogens) (Fig 1). Staphylococcus aureus was rarely
identified (28 isolates, 1.6% of all identified pathogens). Fungi were cultured from 137 of the cultures (7.8%), with all of them found to be Candida spp. On the species level, Candida albicans was detected in 93 cases (67.9% of all candida isolates) and Candida non-albicans in 44 cases (32.1% of all Candida isolates) (Fig 1).

Contemporary resistance profiles of E. coli and Enterococcus spp. isolates were analyzed using microbiological data between 2006 and 2015 (Table 1). Overall, 27/77 (35.1%) extended-spectrum beta-lactamase (ESBL)-producing E. coli strains were identified. Quinolone resistance was frequent in the E. coli isolates (24/77 isolates, 31.2%), whereas resistance to carbapenems was not detected. Isolated enterococci exhibited vancomycin resistance in 14/137 cases (10.2%), Ampicillin resistance among enterococci was observed in 45/137 isolates (32.9%), resistance against carbapenems in 53/137 isolates (38.7%) and against linezolid in 2/137 isolates (1.5%, both E. faecalis) (Table 1).

Resistance to empiric antibiotic therapy

To identify individual risk factors for AC with resistant microorganisms and therapeutic failure, we analyzed the first AC episode of 83 patients seen at the JUH between 2006 and 2012.
The pathogen spectrum of the 209 microorganisms, which were isolated from these patients was representative of the overall spectrum described above, and is shown in Fig 2A. Fifty-five (66%) of the patients were male, and the median hospital stay was 8 days. Fifty-two patients (63%) had a previous ERCP, 51 (61%) had a prior papillotomy and 44 (53%) had undergone BD stenting. Fifty-five patients (66%) showed signs of biliary obstruction when microbiological sampling was performed, with choledocholithiasis being the most common cause of obstruction in 46 patients (55% of all patients) (Table 2). Multi-drug resistant (MDR) bacteria were isolated from the bile of 24 (29%) patients, including ESBL-producing Enterobacteriaceae (13 patients: 11 E. coli and 2 Klebsiella pneumoniae), vancomycin-resistant Enterococcus (VRE) (7 patients), and Pseudomonas aeruginosa (4 patients). Among the isolated ESBL-producing Enterobacteriaceae, 9 were non-susceptible to at least one agent in at least 3 antimicrobial categories (MDR) and were non-susceptible to at least 1 agent in all but two or fewer categories (XDR) according to the interim definition of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) [21]. The identified Pseudomonas spp. fulfilled the MDR criteria of the ECDC/CDC.

Blood cultures were obtained in parallel with bile sampling for 70 of the 83 patients (84%), with positive results obtained for 28 patients (40% of all blood cultures) and negative results obtained for 42 patients (60% of all blood cultures). Two blood cultures had polymicrobial contents (E. coli and E. faecium). Fifteen out of the 28 positive cultures displayed identical pathogens in both the blood and bile cultures (54% of all positive blood cultures), including 5 with an identical pathogen and resistance pattern, and in cases of polymicrobial bile culture, 10 cases showed at least one pathogen in the blood culture that was isolated from the bile. The pathogens that were isolated from blood cultures included E. coli in 14 patients (50% of all positive blood cultures), Enterococcus spp. in 5 patients (18% of all positive blood cultures), including E. faecium (n = 3), E. faecalis (n = 1) and E. durans (n = 1), Klebsiella spp. for 4 cases (14%), Staphylococcus hominis in 3 cases (11%), indicating possible skin contamination, and one case each (4%) of methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa and Streptococcus pneumoniae. Overall, MDR bacteria were isolated from the blood of 5 patients (7% of patients for whom blood cultures were performed; 6% of all patients) including ESBL-producing E. coli (3 patients), MRSA (1 patient) and Pseudomonas aeruginosa (1 patient). In addition, fungal bloodstream infection by Candida albicans was detected in one patient from whom Candida was not isolated from the bile.

Twenty-nine patients (35%) received antibiotic monotherapy, 38 (46%) received antibiotic combination therapy, and 16 (19%) did not receive any empiric antibiotic therapy. Ceftriaxone was the antimicrobial substance most frequently used for empiric therapy in 36 of the 83

| Antibiotic          | E. coli (N = 77) | Enterococci (N = 137) |
|---------------------|-----------------|-----------------------|
| Ceftriaxone         | 32.5%           | n.a.                  |
| Ampicillin/Sulbactam| 51.9%           | 32.9%                 |
| Piperacillin/Tazobactam | 46.8%         | n.a.                  |
| Quinolones          | 31.2%           | 32.8%*                |
| Carbapenems         | 0%              | 38.7%                 |
| Vancomycin          | n.a.            | 10.2%                 |
| Gentamicin          | n.a.            | 32.2%                 |
| Linezolid           | n.a.            | 1.5%                  |

*data available for 58 out of 137 isolates; n.a.: not applicable.

doi:10.1371/journal.pone.0169900.t001

The pathogen spectrum of the 209 microorganisms, which were isolated from these patients was representative of the overall spectrum described above, and is shown in Fig 2A. Fifty-five (66%) of the patients were male, and the median hospital stay was 8 days. Fifty-two patients (63%) had a previous ERCP, 51 (61%) had a prior papillotomy and 44 (53%) had undergone BD stenting. Fifty-five patients (66%) showed signs of biliary obstruction when microbiological sampling was performed, with choledocholithiasis being the most common cause of obstruction in 46 patients (55% of all patients) (Table 2). Multi-drug resistant (MDR) bacteria were isolated from the bile of 24 (29%) patients, including ESBL-producing Enterobacteriaceae (13 patients: 11 E. coli and 2 Klebsiella pneumoniae), vancomycin-resistant Enterococcus (VRE) (7 patients), and Pseudomonas aeruginosa (4 patients). Among the isolated ESBL-producing Enterobacteriaceae, 9 were non-susceptible to at least one agent in at least 3 antimicrobial categories (MDR) and were non-susceptible to at least 1 agent in all but two or fewer categories (XDR) according to the interim definition of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) [21]. The identified Pseudomonas spp. fulfilled the MDR criteria of the ECDC/CDC.

Blood cultures were obtained in parallel with bile sampling for 70 of the 83 patients (84%), with positive results obtained for 28 patients (40% of all blood cultures) and negative results obtained for 42 patients (60% of all blood cultures). Two blood cultures had polymicrobial contents (E. coli and E. faecium). Fifteen out of the 28 positive cultures displayed identical pathogens in both the blood and bile cultures (54% of all positive blood cultures), including 5 with an identical pathogen and resistance pattern, and in cases of polymicrobial bile culture, 10 cases showed at least one pathogen in the blood culture that was isolated from the bile. The pathogens that were isolated from blood cultures included E. coli in 14 patients (50% of all positive blood cultures), Enterococcus spp. in 5 patients (18% of all positive blood cultures), including E. faecium (n = 3), E. faecalis (n = 1) and E. durans (n = 1), Klebsiella spp. for 4 cases (14%), Staphylococcus hominis in 3 cases (11%), indicating possible skin contamination, and one case each (4%) of methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa and Streptococcus pneumoniae. Overall, MDR bacteria were isolated from the blood of 5 patients (7% of patients for whom blood cultures were performed; 6% of all patients) including ESBL-producing E. coli (3 patients), MRSA (1 patient) and Pseudomonas aeruginosa (1 patient). In addition, fungal bloodstream infection by Candida albicans was detected in one patient from whom Candida was not isolated from the bile.

Twenty-nine patients (35%) received antibiotic monotherapy, 38 (46%) received antibiotic combination therapy, and 16 (19%) did not receive any empiric antibiotic therapy. Ceftriaxone was the antimicrobial substance most frequently used for empiric therapy in 36 of the 83

| Antibiotic          | E. coli (N = 77) | Enterococci (N = 137) |
|---------------------|-----------------|-----------------------|
| Ceftriaxone         | 32.5%           | n.a.                  |
| Ampicillin/Sulbactam| 51.9%           | 32.9%                 |
| Piperacillin/Tazobactam | 46.8%         | n.a.                  |
| Quinolones          | 31.2%           | 32.8%*                |
| Carbapenems         | 0%              | 38.7%                 |
| Vancomycin          | n.a.            | 10.2%                 |
| Gentamicin          | n.a.            | 32.2%                 |
| Linezolid           | n.a.            | 1.5%                  |

*data available for 58 out of 137 isolates; n.a.: not applicable.

doi:10.1371/journal.pone.0169900.t001
Fig 2. Pathogen spectra and in vitro resistance to empiric first-line therapy. 209 pathogens were isolated from bile in 83 episodes of acute cholangitis. (A) The proportion of isolated pathogens is given as the percentage of isolates (left panel) and as the percentage of patients (right panel). (B) In vitro susceptibility of isolated pathogens is given for patients treated with empiric monotherapy (left panel) or with empiric combination therapy (right panel). Black bars represent the number of patients, in whom all isolated pathogens were susceptible; the white bars represent the number of patients in whom one or more of the isolated pathogens are resistant against the antibiotic agent. For antibiotic substances, which were used in more than 10 episodes, percentage of fully covered pathogen spectra are shown.

doi:10.1371/journal.pone.0169900.g002
patients (43%), followed by ciprofloxacin, metronidazole, piperacillin/tazobactam and vancomycin (Fig 2B). According to the in vitro resistance profiles, isolated pathogens were fully susceptible to the empiric monotherapy regimens in 7/29 (24%) patients and to the empiric combination therapy regimens in 11/38 (29%) resulting in overall 65/83 (78%) patients who did not receive an empiric antibiotic before ERCP, covering the isolates from bile. Overall, the initial empiric therapy was changed according to the resistance patterns observed in 40 (60%) of 67 patients. The switching of antibiotic therapy due to in vitro resistance by an additionally detected pathogen was frequently observed, occurring in 53% of the patients treated with ceftriaxone (N = 19; 23% of patients) and in 45% of the patients treated with ciprofloxacin (N = 10; 12% of patients) compared with 10% of the patients treated with piperacillin/tazobactam (N = 8; 7% of all patients) and 33% of the patients treated with vancomycin (N = 2; 2% of patients).

### Risk factors for AC with resistant bacteria

Twenty-four (29%) patients with AC had MDR bacteria in bile, including ESBL-producing Enterobacteriaceae, VRE, and Pseudomonas aeruginosa. AC with MDR bacteria was associated with the male sex (83% vs. 59%; \( P = 0.043 \)), being hospitalized for more than 48 h (58% vs. 32%; \( P = 0.047 \)), prior biliary stenting (75% vs. 44%, \( P = 0.025 \)) and exposure to antibiotics within 14 days of bile sampling for cultures (42% vs. 17%; \( P = 0.012 \)). In contrast, BD stones, biliary obstruction and previous ERCP were not identified as significant risk factors for MDR pathogens according to univariate analysis (Table 3), and no association with diabetes, chronic lung disease or cirrhosis of the liver was found (data not shown). Expanding the analysis to also include patients with Candida in the bile (N = 15), patients with biliary MDR bacteria or Candida more often had cholangiocarcinoma (27.0% vs. 8.7%, \( P = 0.039 \)). Because enterococci show intrinsic resistance to 3GC (which are recommended for calculated antibiotic therapy by

---

**Table 2. Baseline patient characteristics.**

| Characteristic                        | N  = 83 |
|---------------------------------------|---------|
| Male gender (n)                       | 55 (66.3%) |
| Admitted to ICU (n)                   | 10 (12%) |
| Age (years)                           | 69 (60; 76) |
| Length of stay (days)                 | 8 (6; 18) |
| WBC \( \times 10^{3}/\mu l \)          | 10.1 (7.6; 13.2) |
| CRP (mg/l)                            | 85.8 (41.3; 167.6) |
| ALT (\( \mu \text{mol/l}\times s \)) | 1.02 (0.66; 1.97) |
| AST (\( \mu \text{mol/l}\times s \)) | 1.11 (0.63; 1.86) |
| AP (\( \mu \text{mol/l}\times s \))  | 4.26 (2.15; 7.18) |
| GGT (\( \mu \text{mol/l}\times s \)) | 7.44 (2.73; 12.35) |
| Bilirubin (\( \mu \text{mol/l} \))    | 47 (18.5; 103.75) |
| Prior ERCP                            | 52 (63%) |
| Prior biliary stent                   | 44 (53%) |
| Prior papillotomy                     | 51 (61%) |
| Primary sclerosing cholangitis        | 5 (6%)  |
| Choledocholithiasis                   | 46 (55%) |
| Biliary cancer                        | 14 (17%) |
| Biliary obstruction at ERCP           | 55 (66%) |

Data are presented as absolute numbers and percentages or as median values with first and third quartiles.

DOI: 10.1371/journal.pone.0169900.0002
the current guidelines), further analysis was performed by including all patients from whose bile samples enterococci were cultured in the cohort of patients with MDR bacteria. In this analysis, only a prior biliary stenting (60% vs. 39%, \( p = 0.025 \)) remained a significant risk factor for the occurrence of MDR pathogens, including enterococci (S1).

Multivariate analysis (including sex, nosocomial acquisition, prior BD stenting and antibi-otic exposure), performed using backward exclusion, revealed that a prior biliary stenting was the only independent predictor of AC with MDR bacteria (OR = 3.808; 95% CI 1.323–10.960, \( p = 0.013 \)) or AC with enterococci (OR = 3.694; 95% CI 1.408–9.695; \( p = 0.008 \)).

**Course and outcome of AC with MDR bacteria**

There were no significant differences in the length of hospital stay of patients with AC by MDR bacteria compared with that of patients without these pathogens (12.5 days vs. 8 days; \( p = 0.18 \)). There were two in-hospital deaths following AC, one involving a patient with AC with Enterococci and Staphylococcus aureus and the other a patient with AC with E. coli and C. albicans. Notably, patients with biliary MDR bacteria had higher levels of cholestasis, as shown by their alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGT) levels, but had lower white-blood-cell counts, indicating less severe inflammation (Table 3). However, patients whose bile cultures were positive for MDR pathogens more often had

---

**Table 3. Risk factors for acute cholangitis with multi-resistant pathogens* in the bile.**

|                                | With multi-resistant pathogens* (n = 24) | Without multi-resistant pathogens (N = 59) | P-value |
|--------------------------------|----------------------------------------|-------------------------------------------|---------|
| Sex male (n)                   | 20 (83.3%)                             | 35 (59.3%)                                | \( p = 0.043 \) |
| Admitted to ICU (n)            | 2 (8.3%)                               | 8 (13.6%)                                 | \( p = 0.401 \) |
| Age (years)                    | 67.5 (61.75; 71.5)                     | 69 (59.79)                                 | \( p = 0.527 \) |
| Length of stay (days)          | 12.5 (6.75; 21)                        | 8 (6; 15.5)                                | \( p = 0.177 \) |
| Hospital associated (n)        | 14 (58.3%)                             | 14 (32.2%)                                | \( p = 0.047 \) |
| WBC (GPT/l)                    | 8.9 (4.7; 11.5)                        | 10.5 (7.0; 15.1)                          | \( p = 0.014 \) |
| CRP (mg/l)                     | 95.7 (67.7; 170.8)                     | 79.9 (34.7; 166.4)                        | \( p = 0.241 \) |
| ALT (\( \mu \)mol/l\times s)   | 0.98 (0.58; 1.59)                      | 1.07 (0.71; 2.12)                         | \( p = 0.248 \) |
| AST (\( \mu \)mol/l\times s)   | 0.93 (0.56; 1.70)                      | 1.19 (0.64; 2.30)                         | \( p = 0.490 \) |
| AP (\( \mu \)mol/l\times s)    | 7.21 (4.21; 9.47)                      | 3.20 (1.99; 5.69)                         | \( p = 0.021 \) |
| GGT (\( \mu \)mol/l\times s)   | 11.10 (3.82; 18.18)                    | 5.88 (2.58; 11.1)                         | \( p = 0.040 \) |
| Bilirubin (\( \mu \)mol/l)     | 40.2 (14.5; 98.3)                      | 49.5 (21.0; 121.8)                        | \( p = 0.657 \) |
| Prior ERCP                     | 19 (79.2%)                             | 33 (55.9%)                                | \( p = 0.092 \) |
| Prior biliary stent            | 18 (75.0%)                             | 26 (44.1%)                                | \( p = 0.025 \) |
| Prior papillotomy              | 19 (79.2%)                             | 32 (54.2%)                                | \( p = 0.072 \) |
| PSC                            | 0                                      | 5 (8.5%)                                  | \( p = 1.000 \) |
| Choledocholithiasis            | 10 (41.7%)                             | 36 (61.0%)                                | \( p = 0.145 \) |
| Biliary cancer                 | 7 (29.2%)                              | 7 (11.9%)                                 | \( p = 0.102 \) |
| Biliary obstruction at ERCP    | 19 (79.2%)                             | 36 (61.0%)                                | \( p = 0.781 \) |
| Previous cholecystectomy       | 12 (50.0%)                             | 24 (40.7%)                                | \( p = 0.296 \) |
| Immunosuppression              | 8 (33.3%)                              | 8 (13.6%)                                 | \( p = 0.063 \) |
| Antibiotics 14 days before admission | 10 (41.7%)                              | 9 (15.3%)                                | \( p = 0.012 \) |
| Positive blood cultures        | 14 (58.3%)                             | 14 (23.7%)                                | \( p = 0.019 \) |
| Blood cultures with MDR bacteria or fungi** | 5 (20.8%)                          | 1 (1.7%)                                  | \( p = 0.007 \) |

* ESBL-producing Enterobacteriaceae (N = 13), VRE (N = 7) or Pseudomonas aeruginosa (N = 4) in the bile.
** ESBL-producing Enterobacteriaceae (N = 3), MRSA (N = 1), Pseudomonas aeruginosa (N = 1) or Candida albicans (N = 1) in the blood.

doi:10.1371/journal.pone.0169900.003
concomitant positive blood cultures (58% vs. 24%; \( P = 0.019 \)). In addition, bloodstream infec-
tion by MDR bacteria or Candida spp. was diagnosed significantly more often in patients with 
MDR pathogens in the bile (21% of patients; 36% of blood culture positive patients) compared 
with patients without biliary MDR pathogens (2% of patients; 7% of blood culture positive 
patients) (Table 3).

**Discussion**

In this study, we found a high proportion of AC due to enterococci and E. coli in two German 
university hospitals, which is consistent with the findings of other recent studies [8,22]. The 
pathogen profile remained stable over different periods. Due to the polymicrobial culture 
results and the high rate of MDR bacteria in the bile (24/83; 29%), empiric antibiotic treatment 
was only successful in covering the resistant microorganisms in only a minority of cases (20/ 
67; 30%). We identified previous biliary stenting as the most relevant independent risk factor 
for developing AC with MDR bacteria or enterococci, which increased the odds by more than 
three-fold. These results are consistent with those of a recently published study by Schneider 
et al. [23], in which biliary stenting was identified as the major risk factor for developing AC 
with 3GC-resistant bacteria. Because biliary stents are generally changed regularly, biliary 
stenting can be considered a surrogate marker for previous hospitalization, which is known to 
affect both antibiotic exposure and antibiotic resistance profiles [24]. Indeed, our univariate 
analysis confirmed that antibiotic exposure and hospitalization are risk factors for MDR bacte-
ria occurring in bile.

A recent prospective German study in 120 patients found a high rate of biliary stent polymi-
bacterial colonization, in 96% of the patients, even in the absence of clinical signs of cholangitis. 
The most frequently observed pathogens were Enterococci (79.3%) and Enterobacteriaceae 
(73.7%). Candida spp. were isolated from 55.9% of the patients [13]. Due to these high rates of 
bacterial colonization, the authors suggested microbiological testing of explanted biliary stents 
as a helpful diagnostic procedure to identify the causative pathogens of AC [13]. Schneider 
et al. found that Enterococci (22%) Klebsiella spp. (10%) and Candida spp. (8%) were the most 
common organisms colonizing indwelling stents, with a predominance of Gram-positive bac-
teria and fungi after a short indwelling period and an increasing proportion of Gram-negative 
bacteria after an indwelling period of more than 60 days [25]. Patients undergoing biliodiges-
tive anastomosis had a higher risk of bacterial and fungal colonization of the BD when under-
going preoperative biliary drainage [26] and had a higher risk of AC with MDR bacteria in the 
case of preoperative biliary duct stenting [27].

The results of our study argue against the use of 3GC as a first-line antibiotic therapy in 
patients at risk for MDR-associated AC because they do not cover ESBL-producing Enterobac-
teriaceae or enterococci, which are frequently isolated from the bile of patients with AC. In sev-
eral studies performed throughout the world, the percentage of MDR bacteria isolated from 
the bile of patients with AC exceeded 20 to 30% [18], and intrinsically 3GC-resistant enteroc-
cocci are isolated in 36–74% of AC episodes [8,28,22], particularly those involving patients 
with underlying biliary disease, e.g., sclerosing cholangitis [8], or a liver transplant [28]. In our 
study, empiric treatment with 3GC did not cover the isolated pathogens in more than 50% of 
patients and required the escalation of therapy. Metronidazole was added as part of an empiric 
combination therapy for 19 patients; however, based on the bile culture analyses, it was dis-
ensable in 18 of these cases. In agreement with Lee et al., who found that adding metronida-
azole to the treatment regimen did not improve the outcome of severe AC [29], it appears 
reasonable to not recommend adding metronidazole as a first-line therapy if emergency drain-
age of the bile duct can be performed. Based on our data, replacing 3GC with piperacillin/
tazobactam as the empiric antimicrobial therapy would reduce the rate of initially inappropriate treatment for \textit{E. coli} infections from 45–56% to 3–29% and would cover \textit{E. faecalis} strains (22–44%), as well as anaerobes in the overall cohort, but would likely be insufficient for patients with prior biliary stenting.

Our study has several limitations. First, bile cultures do not discriminate colonization from causative infection and because MDR is often associated with a loss of microbial fitness, one may question the clinical relevance of identifying MDR bacteria in bile. However, our study shows that patients with MDR bacteria in the bile more often have concomitant bloodstream infections in general and bloodstream infections by MDR pathogens or fungi in particular, which supports the notion of providing broad-spectrum antibiotic therapy to at-risk patients. Second, the bile samples were obtained primarily by ERCP and may have been contaminated by the autologous oral and duodenal microflora. Although routine bile sampling during ERCP is not generally recommended by current guidelines, it is widely performed in clinical practice in Germany as it allows the identification of pathogens and their antibiotic susceptibility providing a rationale for antibiotic therapy in patients at risk for MDR pathogens [30,31]. This approach is justified by the arguments that the highest bacterial burden during AC is suspected to be in the biliary system, that blood cultures show a lower sensitivity than bile cultures in AC but— if positive—are in high concordance with the corresponding bile cultures, and that even if duodenal contamination may play a role—the presence of MDR gut bacteria may itself impose a risk factor for ascending AC by MDR. Third, because this was a retrospective study, the selection of empiric antibiotic regimens and the reasons for escalation of therapy were diverse. Fourth, because no significant short-term mortality occurred after AC in our study—even in the absence of antibiotic therapy—there were no hard end-points to prove the failure of antibiotic therapy. Recent studies that could show an association between the microbial bile profile and survival were performed retrospectively and identified MDR bacteria or fungi as risk factors for mortality in patients with malignant biliary obstruction [32], primary sclerosing cholangitis [30] or a liver transplant [28,33]. Whether this association was causative and how it can be addressed by appropriate treatment is unknown. To clarify this important diagnostic question, prospective studies involving appropriate bacterial typing are urgently needed.

Based on the \textit{in vitro} resistance data presented in this study, patients with biliary stents who develop AC should receive empiric therapy covering enterococci and ESBL-producing \textit{Enterobacteriaceae} because these patients are at greater risk for bacteremia. Controlled prospective trials are needed to determine whether improved antibiotic therapy translates into better outcomes for patients with AC.

**Supporting Information**

**S1 Table. Risk factors for infection with multi-resistant pathogens or enterococci**

| Pathogens and First-Line Therapy in Cholangitis |
|-----------------------------------------------|
| Supporting Information                        |
| **S1 Table. Risk factors for infection with multi-resistant pathogens or enterococci** | |
| * ESBL-producing Enterobacteriaceae (N = 13); VRE (N = 7), Pseudomonas aeruginosa (N = 4), MRSA (N = 0) and enterococci (N = 58), ** ESBL-producing Enterobacteriaceae (N = 3), MRSA (N = 1), Pseudomonas aeruginosa (N = 1) or Candida albicans (N = 1) in blood. | (DOCX) |

**Author Contributions**

**Conceptualization:** AS TB.

**Data curation:** PAR CL NL TB.

**Formal analysis:** PAR DT.
Investigation: PAR DT MB BL CL NL AS TB.
Methodology: PAR AS CL NL TB.
Project administration: AS TB.
Resources: PAR MB BL CL NL.
Software: PAR TB.
Supervision: AS TB.
Validation: PAR TB.
Visualization: PAR TB.
Writing – original draft: PAR TB.
Writing – review & editing: PAR DT MB BL CL NL AS TB.

References
1. Wada K, Takada T, Kawarada Y, Nimura Y, Miura F, Yoshida M, et al. Diagnostic criteria and severity assessment of acute cholangitis: Tokyo Guidelines. J Hepatobiliary Pancreat Surg. 2007; 14: 52–58. doi: 10.1007/s00534-006-1156-7 PMID: 17252297
2. Scott-Conner CE, Grogan JB. The pathophysiology of biliary obstruction and its effect on phagocytic and immune function. J Surg Res. 1994; 57: 316–336. doi: 10.1006/jsre.1994.1151 PMID: 8028341
3. Sung JY, Costerton JW, Shaffer EA. Defense system in the biliary tract against bacterial infection. Dig Dis Sci. 1992; 37: 689–696. PMID: 1563308
4. Lee JG. Diagnosis and management of acute cholangitis. Nat Rev Gastroenterol Hepatol. 2009; 6: 533–541. doi: 10.1038/nrgastro.2009.126 PMID: 19652653
5. Usenko AY, Yareshko VG, Nichitaioy ME, Mikheyev YA, Andreyeshchev SA. [TG13: THE UPDATED TOKYO’S CLINICAL RECOMMENDATIONS FOR TREATMENT OF AN ACUTE CHOLANGITIS AND CHOLECYSTITIS]. Klin Khirurhi a Minist Okhorony Zdorovia Ukrainy Nauk Tovaryshtvo Khirurhi Ukrainy. 2015; 5–10.
6. Zimmer V, Lammert F. Acute Bacterial Cholangitis. Viszeralmedizin. 2015; 31: 166–172. doi: 10.1159/000439065 PMID: 26468310
7. Lee F, Ohanian E, Rheem J, Laine L, Che K, Kim JJ. Delayed endoscopic retrograde cholangiopancreatography is associated with persistent organ failure in hospitalised patients with acute cholangitis. Aliment Pharmacol Ther. 2015; 42: 212–220. doi: 10.1111/apt.13253 PMID: 25997554
8. Voigtländer T, Leuchs E, Vonberg R-P, Solbach P, Manns MP, Suerbaum S, et al. Microbiological analysis of bile and its impact in critically ill patients with secondary sclerosing cholangitis. J Infect. 2015; 70: 483–490. doi: 10.1016/j.jinf.2015.01.013 PMID: 26569761
9. Gargouri D, Ouakaa-Kchaou A, Kochlef A, Elloumi H, Bibani N, Trad D, et al. Microbiological study and antimicrobial susceptibility of bile in biliary therapeutic endoscopy. Tunis Med. 2015; 93: 602–605.
10. Weber A, Schneider J, Wagenpeil S, Winkle P, Riedel J, Wantia N, et al. Spectrum of pathogens in acute cholangitis in patients with and without biliary endoprosthesis. J Infect. 2013; 67: 111–121. doi: 10.1016/j.jinf.2013.04.008 PMID: 239803487
11. Bornscheuer T, Schmiedel S. Calculated Antibiosis of Acute Cholangitis and Cholecystitis. Viszeralmedizin. 2014; 30: 297–302. doi: 10.1159/000368335 PMID: 26535043
12. Csendes A, Mitru N, Maluenda F, Diaz JC, Burdiles P, Csendes P, et al. Counts of bacteria and pyocites of choledochal bile in controls and in patients with gallstones or common bile duct stones with or without acute cholangitis. Hepatogastroenterology. 1996; 43: 800–806. PMID: 8884293
13. Lübbert C, Wendt K, Feisthammel J, Moter A, Lippmann N, Busch T, et al. Epidemiology and Resistance Patterns of Bacterial and Fungal Colonization of Biliary Plastic Stents: A Prospective Cohort Study. PloS One. 2016; 11: e0155479. doi: 10.1371/journal.pone.0155479 PMID: 27171497
14. Park JW, Lee JK, Lee KT, Lee KH, Sung YK, Kang C-I. How to interpret the bile culture results of patients with biliary tract infections. Clin Res Hepatol Gastroenterol. 2014; 38: 300–309. doi: 10.1016/j.clinre.2014.02.005 PMID: 24674840
15. Tanaka A, Takada T, Kawarada Y, Nimura Y, Yoshida M, Miura F, et al. Antimicrobial therapy for acute cholangitis: Tokyo Guidelines. J Hepatobiliary Pancreat Surg. 2007; 14: 59–67. doi: 10.1007/s00534-006-1157-6 PMID: 17252298

16. Sun Z, Zhu Y, Zhu B, Xu G, Zhang N. Controversy and progress for treatment of acute cholangitis after transplantation. Endoscopy. 2013; 45: 890–896. doi: 10.1055/s-0033-1344713 PMID: 24165814

17. Park TY, Choi JS, Song TJ, Do JH, Choi S-H, Oh H-C. Early oral antibiotic switch compared with conventional intravenous antibiotic therapy for acute cholangitis with bacteremia. Dig Dis Sci. 2014; 59: 2790–2796. doi: 10.1007/s10620-014-3233-0 PMID: 24898101

18. Kwon JS, Han J, Kim TW, Oh JH, Kwon HH, Jung JT, et al. Changes in causative pathogens of acute cholangitis and their antimicrobial susceptibility over a period of 6 years. Korean J Gastroenterol. 2014; 63: 299–307. PMID: 24870302

19. Annual epidemiological report 2014—Antimicrobial resistance and healthcare-associated infections [Internet]. Available: ecdc.europa.eu

20. Karlmeter G, Brown DFJ, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, et al. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother. 2003; 52: 145–148. doi: 10.1093/jac/dkg312 PMID: 1287738

21. Magiorakos A-P, Srinivasan A, Carey RB, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18: 268–281. doi: 10.1111/j.1469-0691.2011.03570.x PMID: 21793988

22. Basissoukas P, Vezaakis A, Zarkotou O, Fragulidis G, Themeli-Digalaki K, Rizos S, et al. Isolated microorganisms in plastic biliary stents placed for benign and malignant diseases. Ann Gastroenterol. 2014; 27: 399–403.

23. Schneider J, De Waha P, Hapfelmeier A, Feihl S, Römmler F, Schlag C, et al. Risk factors for increased antimicrobial resistance: a retrospective analysis of 309 acute cholangitis episodes. J Antimicrob Chemother. 2014; 69: 519–525. doi: 10.1093/jac/dkt373 PMID: 24084640

24. Doi Y, Park YS, Rivera JL, Adams-Haduch JM, Hingwe A, Sordillo EM, et al. Community-associated extended-spectrum β-lactamase-producing Escherichia coli infection in the United States. Clin Infect Dis Off Publ Infect Dis Soc Am. 2013; 56: 641–648.

25. Schneider J, Hapfelmeier A, Fremd J, Schenk P, Obermeier A, Burgkart R, et al. Biliary endoprosthesis: a prospective analysis of bacterial colonization and risk factors for sludge formation. PloS One. 2014; 9: e110112. doi: 10.1371/journal.pone.0110112 PMID: 25314593

26. Jethwa P, Breuning E, Bhati C, Buckles J, Mirza D, Bramhall S. The microbiological impact of pre-operative biliary drainage on patients undergoing hepato-biliary-pancreatic (HPB) surgery. Aliment Pharmacol Ther. 2007; 25: 1175–1180. doi: 10.1111/j.1365-2036.2007.03289.x PMID: 17451563

27. Cammann S, Timrott K, Vonberg R-P, Vondrak FWR, Schrem H, Suerbaum S, et al. Cholangitis in the postoperative course after biliodigestive anastomosis. Langenbecks Arch Surg Dtsch Ges Chir. 2016; 329: 140–147.

28. Gotthardt DN, Weiss KH, Rupp C, Bode K, Eckerle I, Rudolph G, et al. Bacteriobilia and fungibilia are associated with outcome in patients with endoscopic treatment of biliary complications after liver transplantation. Endoscopy. 2013; 45: 890–896. doi: 10.1055/s-0033-1344713 PMID: 24165814

29. Lee JK, Park CW, Lee SH, Kang HW, Kwon JH, Kim JH, et al. Changes in causative pathogens of acute cholangitis and their antimicrobial susceptibility over a period of 6 years. Korean J Gastroenterol. 2014; 63: 299–307. PMID: 24870302

30. Rupp C, Bode KA, Chahoud F, Wannhoff A, Friedrich K, Weiss K-H, et al. Risk factors and outcome in patients with primary sclerosing cholangitis with persistent biliary candidiasis. BMC Infect Dis. 2014; 14: 562. doi: 10.1186/s12879-014-0562-6 PMID: 25338733

31. Negm AA, Schott A, Vonberg R-P, Weismueller TJ, Schneider AS, Kubicka S, et al. Routine bile collection for microbiological analysis during cholangiography and its impact on the management of cholangitis. Gastrointest Endosc. 2010; 72: 284–291. doi: 10.1016/j.gie.2010.02.043 PMID: 20541201

32. Haag G-M, Herrmann T, Jaeger D, Stremmel W, Schemmer P, Sauer P, et al. Outcomes and risk factors for cancer patients undergoing endoscopic intervention of malignant biliary obstruction. BMC Gastroenterol. 2015; 15: 171. doi: 10.1186/s12876-015-0399-7 PMID: 26637394

33. Lübbert C, Becker-Rux D, Rodloff AC, Laudi S, Busch T, Bartels M, et al. Colonization of liver transplant recipients with KPC-producing Klebsiella pneumoniae is associated with high infection rates and excess mortality: a case-control analysis. Infection. 2014; 42: 309–316. doi: 10.1007/s15010-013-0547-3 PMID: 24217959