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Analysis of Bile Colonization and Intestinal Flora may Improve Management in Liver Transplant Recipients Undergoing ERCP

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Background: Immunosuppression, denervation of biliary tract, and presence of biliary strictures favor colonization of bile with microorganisms after liver transplantation. Little is known about spectrum and antibiotic susceptibility of this colonization.

Material/Methods: Bile and feces were collected prospectively from 38 patients who underwent endoscopic retrograde cholangiopancreaticography after liver transplantation. Samples were analyzed for colonization and antibiotic susceptibility.

Results: From the 38 tested bile samples, 86.6% tested positive. Of those, 26 (78.8%) were polymicrobial. Of isolated bile samples, 52 (64.2%) were gram-positive, 22.2% were gram-negative, and 13.6% revealed *Candida albicans*. Most detectable gram-positive bacteria were *Enterococcus faecium*. Most detectable gram-negative bacteria were *E. coli* and *Klebsiella pneumonia*. Our analyses revealed high resistance rates of the isolates. Only 55.6% of isolates were sensitive to ciprofloxacin, 54% were sensitive to piperacillin/tazobactam, and 60.3% were sensitive to imipenem. High susceptibility rates were found for linezolid and vancomycin (72.9% and 72.6%, respectively). We found a high correlation between microorganisms found in bile and those isolated from stool.

Conclusions: Bile of liver transplant recipients is frequently colonized with microorganisms. The starting point of this colonization is usually the intestine. Systematic analysis of bile colonization during endoscopic interventions on biliary tracts of liver transplant recipients might help to select effective prophylactic antibiotic regimes as well as to facilitate the choice of suitable antimicrobial therapy in case of septic complications.

MeSH Keywords: Bile Duct Diseases • Cholangiopancreatography, Endoscopic Retrograde • Liver Transplantation

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Background

Biliary complications after liver transplantation (LT) are common and remain a major source of morbidity; thus, they significantly influence patient and organ survival [1]. In recent years, endoscopic retrograde cholangiopancreatography (ERCP) has superseded surgery as the gold standard in management of bile duct complications after LT [2]. However, ERCP still is an invasive tool and may have serious complications such as pancreatitis, perforation, bleeding, and septic complications [2,3].

Infectious complications are among the most common causes of death after ERCP [4,5]. Septic complications after ERCP include bacteremia, sepsis, ascending cholangitis, and liver abscess. A potential focus for infectious complications may be bactibilia.

Although bile is sterile in healthy individuals [6,7], bactibilia is frequently found in patients after LT. Additional risk factors for colonization of bile include the presence of foreign bodies such as stones and stents [8,9].

Immunosuppression and occurrence of biliary strictures after LT favor colonization of bile with microorganisms. Little is known about the clinical relevance of this colonization, but preliminary data show that colonization of bile with bacteria and fungi is associated with development of septic complications after endoscopic interventions on the bile duct [10]. However, recent antibiotic prophylaxis of ERCP-induced septic complications is based on recommendations in the non-transplant setting. Furthermore, there are currently no guidelines for the treatment of septic complications after endoscopic biliary interventions in liver transplant recipients. This is especially important because most of these patients have had multiple antibiotic therapies in the course of their liver disease.

Therefore, the aim of this study was to evaluate microbiological spectrum and antibiotic resistance of specimens found in bile of liver-transplanted individuals who underwent ERCP, in order to investigate the clinical efficacy of antibiotics administered to prevent infectious complications. Since there is evidence that enteric flora represents a reservoir for ascending cholangitis and further infectious diseases, particularly after LT [11,12], we additionally analyzed enteric flora and the correlation between isolates found in bile and stool cultures in our patient population.

Material and Methods

This prospective study was carried out between May 2011 and September 2013 at the University Hospital of Münster. Thirty-eight patients (25 (65.8%) were male and 13 (34.2%) were female, mean age was 52.2±11.4 years) were enrolled into the study after LT. Table 1 shows patients' characteristics and endoscopic findings. All patients gave written informed consent and treatment was performed in adherence to the guidelines of the Declaration of Helsinki. The trial was approved by the local ethics committee. Indications for ERCP were increasing liver and cholestatic parameters with or without an intra- or extra-hepatic biliary dilatation seen on imaging. All patients were treated with at least 1 immunosuppressant. Twenty-seven (71.1%) were on calcineurin-inhibitor-based immunosuppression (57.9% on tacrolimus and 13.1% on ciclosporin), and eight (21.1%) were on m-TOR-based immunosuppression (13.2% everolimus and 7.9% sirolimus). Two patients (5.3%) were maintained on tacrolimus in combination with everolimus and 1 patient (2.6%) received mycophenolate mofetil only.

Table 1. Demographic and clinical characteristics and endoscopic findings in the 38 LT recipients.

N	38
Male	25 (65.8%)
Female	13 (34.2%)
Age, yr, mean ±SD	52.2±11.4
Etiology of liver disease	
Alcohol	15
HCC	8
Hepatitis B/C virus	5
Cholestatic liver diseases	5
Other	5
Time from transplant to ERCP, months	25.6 (range 0.3–164.2)
Endoscopic findings	
AS	13 (44.8%)
NAS	4 (13.8%)
Biliary leakage	4 (13.8%)
Stones	1 (3.4%)
ITBL	1 (3.4%)
Biliary cast	1 (3.4%)
Non	5 (17.2%)

HCC – hepatocellular carcinoma; AS – anastomotic stricture; NAS – non-anastomotic stricture, ITBL – ischemic type biliary lesion.

Bile was collected either via percutaneous biliary drainage (PTC) in nine patients and during ERCP in 29 patients. Endoscopic retrograde cholangiopancreaticography was performed using a therapeutic duodenoscope (TJF-180V, Olympus Corp., Tokyo, Japan). The bile duct was accessed with wire-guided cannulation

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using a sterile sphincterotome (0.035 inch, THSF-35-480, Cook Medical INC.; Tri-Tome, TRI 20, Cook Medical, Inc.). Bile was aspirated immediately after cannulation of the bile duct before contrast material was administered. Approximately 2–5 mL of bile were collected and immediately submitted to microbiological laboratory analysis in a sterile syringe. Stool samples were also collected from each patient and submitted to the microbiological laboratory in the same way.

Bile was streaked out on Columbia blood agar, McConkey agar, Kimmig agar, and boiled blood agar under increased CO_2 atmosphere (5–10%) to detect aerobic growth. To detect anaerobic microorganisms, we used Schaedler agar and Thioglycolate-Bouillon. All samples were incubated at 35° to 37°C. Plates were examined after 24 h and 48 h. For identification, we used matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, microflex©, Bruker Daltonik GmbH, Bremen). Susceptibility testing was performed by Vitek 2 (bioMerieux©, Nürtingen, Germany).

If bile specimens showed any growth of microorganisms, stool specimens were investigated in the same way. Only microorganisms previously found in bile cultures were identified and tested for antibiotic susceptibility.

Antibiotic prophylaxis

Antibiotics were routinely administered prophylactically in all patients who received ERCP; 18 out of 29 patients (62.1%) received ciprofloxacin (2×750 mg daily), 2 patients (6.9%) received imipenem (3×500 mg daily), and nine patients (31.0%) received piperacillin (3×4 g daily) administered in combination with tazobactam (3×0.5 mg daily). Administration of antimicrobial prophylaxis was started 2 hours before ERCP and was continued for 3 days after intervention. The selection of antibiotics for prophylactic use was made by the responsible attending physician.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 20 for Windows. Data are presented as mean ± standard deviation and percentages for categorical variables. Comparison of data was performed using Fisher's exact test. A 2-sided $p<0.05$ was considered significant.

Results

Endoscopic findings of the 29 patients who received ERCP were anastomotic strictures in 13 cases (44.8%), non-anastomotic strictures in 4 (13.8%), biliary leakage in 4 (13.8%), stones in 1 (3.4%), biliary cast in 1 (3.4%), and normal biliary tree in 5 patients (17.2%).

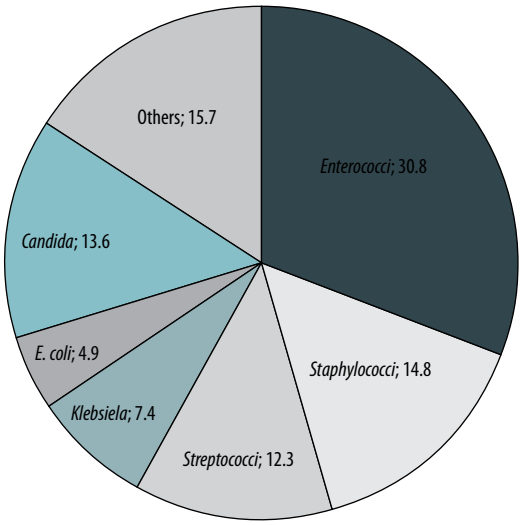

Figure 1. Groups of microorganisms found in bile [%].

From the 38 bile samples obtained, 33 (86.8%) bile cultures tested positive and 5 (13.2%) showed no microbial growth. Seven (21.2%) of the positive bile cultures showed only 1 isolate and 26 (78.8%) were polymicrobial. A total of 81 microorganisms were isolated; 52 (64.2%) gram-positive, 18 (22.2%) gram-negative, and 11 (13.6%) *Candida albicans*. Most common gram-positive isolates were *Enterococcus faecium* (13.6%), *Enterococcus faecalis* (12.3%), *Staphylococcus haemolyticus* (7.4%), and *Staphylococcus epidermidis* (7.4%). Most common gram-negative isolates were *Escherichia coli* (4.9%) and *Klebsiella pneumoniae* (4.9%). There was no difference concerning the microbial spectrum and their resistogram between the isolates from bile collected during ERCP and during PTC. Groups of microorganisms found in bile are shown in Figure 1.

Three patients developed fever in the time between 2 days before and 10 days after ERCP. In all 3, isolates from blood were the same as those found in bile.

According to our results, the only risk factor for bile colonization was a previous endoscopic sphincterotomy, which that was pre-existent in 21 patients (55.3%) ($p=0.012$). Table 2 shows isolates from bile in patients with and without pre-existing endoscopic sphincterotomy. We found no statistical correlation between endoscopic findings and/or stent placement and the colonization of bile ($p>0.05$).

Antibiotic susceptibility in bile

Bacterial isolates were tested for antibiotics typically used for prophylaxis of septic complications after ERCP (ciprofloxacin, imipenem, and piperacillin/tazobactam) and also for vancomycin and linezolid. Susceptibility to ciprofloxacin was found

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Table 2. Microorganisms isolated from bile in patients with and without pre-existing EST.

Isolates	Total	Patients with EST	Patients without EST
Enterococcus faecium	11	8	3
Enterococcus faecalis	10	9	1
Enterococcus avium	4	3	1
Staphylococcus haemolyticus	6	4	2
Staphylococcus epidermidis	6	2	4
Escherichia coli	4	4	0
Klebsiella pneumoniae	4	3	1
Klebsiella oxytoca	2	2	0
Streptococcus parasanguinis	2	0	2
Streptococcus salivarius	2	0	2
Streptococcus anginosus	2	2	0
Streptococcus sanguinis	1	1	0
Streptococcus oralis	1	0	1
Streptococcus pneumoniae	1	0	1
Streptococcus viridans	1	0	1
Stenotrophomonas maltophilia	2	0	2
Hafnia alvei	1	1	0
Bacillus spp.	1	0	1
Lactobacillus gasseri	1	1	0
Lactobacillus rhamnosus	1	0	1
Enterobacter cloacae	1	1	0
Pseudomonas aeruginosa	1	0	1
Pseudomonas stutzeri	1	0	1
Corynebacterium jeikeium	1	1	0
Micrococcus luteus	1	0	1
Kocuria palustris	1	0	1
Proteus mirabilis	1	1	0
Candida	11	10	1
Total	**81**	**53**	**28**

EST – endoscopic sphincterotomy.

in 55.6% of cases, to piperacillin/tazobactam in 54.0%, to imipenem in 60.3%, to vancomycin in 72.6%, and to linezolid in 72.9%. All bacteria with imipenem resistance were gram-negative. One vancomycin-resistant enterococcus (VRE) and 1 bacterium with extended-spectrum beta-lactamase (ESBL) were isolated.

Only 70.8% of isolates collected during ERCP were sensitive to given antibiotic prophylaxis. Had the patients received either linezolid or vancomycin additionally, an additional 25% of bacteria (95.8% in total) would have been covered by the antibiotic therapy.

Of the tested bacteria, 33.3% were resistant to all 3 antibiotics used (ciprofloxacin, imipenem, and piperacillin/combactam) and 41.2% were sensitive to all 3 antibiotics. An add-on administration of linezolid or vancomycin would have covered a total of 90.5% of all resistant bacteria.

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Table 3. Antibiotic resistance of microorganisms.

Antibiotics	Sensitive isolates		Intermediate isolates		Resistant isolates	
	Number	%	Number	%	Number	%
Ciprofloxacin	35	55.6	1	1.6	27	42.9
Piperacillin/tazobactam	34	54.0	4	6.3	25	39.7
Imipenem	38	60.3	1	1.6	24	38.1
Sulfamethoxazole/trimethoprim	15	23.8	1	1.6	47	74.6
Cefotaxime	22	34.9	1	1.6	40	63.5
Erythromycin	13	21.0	4	6.5	45	72.6
Linezolid	43	72.9	–	-–	16	27.1
Vancomycin	45	72.6	–	–	17	27.4

Table 4. Microorganisms isolated from stool.

Isolates	Number	%
Enterococcus faecium	14	32.6
Enterococcus faecalis	7	16.3
Escherichia coli	5	11.6
Candida	6	14.0
Klebsiella pneumoniae	3	7.0
Enterococcus avium	3	7.0
Enterobacter cloacae	1	2.3
Pseudomonas aeruginosa	1	2.3
Staphylococcus aureus	1	2.3
Staphylococcus haemolyticus	1	2.3
Hafnia alvei	1	2.3
Total	**43**	

Stool

A total of 43 microorganisms were isolated from feces (Table 4). Twenty-six (60.5%) were gram-positive, 11 (25.5%) were gram-negative strains, and 6 (14.0%) were *Candida species*. The most common bacteria were *Enterococcus faecium* (32.6%), *Enterococcus faecalis* (16.3%), and *Escherichia coli* (11.6%).

Comparison between bile cultures and stool cultures

In 24.1% of patients, all microorganisms found in bile could also been found in stool. In 75.9% of cases, at least 1 microorganism found in bile was also found in stool and in 24.1% of patients, microorganisms found in bile could not been found in stool.

Discussion

Diagnosis and therapy of biliary complications after LT is a domain of ERCP. However, ERCP is an invasive procedure and can lead to several severe complications, especially if further interventions (such as sphincterotomy, balloon dilatation, and/or endoscopic drain placement) are required. Some of these dreaded complications are septic in nature [12–14]. In our study, we analyzed the spectrum of isolates found in bile of liver transplant recipients during ERCP and tested their sensitivity to given antibiotic prophylaxis.

Septic complications such as cholangitis are dreaded after ERCP in liver transplant population. A starting point for the bile colonization is the intestine. From there, ascending microorganisms can lead to septic complications, especially in case of biliary

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obstruction and/or immunosuppression [15–17]. Negm et al. identified liver transplantation as a risk factor for biliary colonization with microorganisms. In the same study, Negm et al. showed the clinical relevance of collected bile cultures during ERCP in case of complicating cholangitis [8]. Furthermore, Barkholt et al. showed in 1997 that intestinal flora represents a reservoir for ascending cholangitis and other infections, especially after liver transplantation [11].

Our results show a completely different spectrum of microorganisms found in bile of liver transplant recipients in comparison with that found in bile of immunocompetent non-transplant individuals. In our study, in 64.2% of cases we found gram-positive bacteria, in 22.2% gram-negative bacteria, and in 13.5% *Candida*. Most common isolates were *Enterococcus* species, whereas, in the non-transplant setting, more gram-negative bacteria, especially *E. coli* and *klebsiella,* are found [18–21]. Our results confirm former studies that showed a shift in microbial spectrum in bile of immunosuppressed patients after LT towards gram-positive bacteria with a markedly increased incidence of *Enterococci* [8–10]. Those findings emphasize the relevance of analysis of bile isolates in liver transplant recipients with regard to the spectrum and resistogram to select an effective antibiotic prophylaxis for these patients and also to treat potential septic complications after ERCP in liver transplant patients.

The use of only vancomycin or linezolid would have covered nearly 73% of the detected bacteria. The addition of vancomycin or linezolid to the originally administered antibiotics would have covered more than 95% of isolates. This is due to the above-mentioned increased incidence of gram-positive bacteria in the bile of liver transplant patients, especially of *Enterococci*.

In 2006 Millonig et al. found resistance rates to ciprofloxacin, piperacillin / tazobactam, and imipenem of only 19–32% of bacteria isolated from bile of liver transplant recipients [9]. These resistance rates are lower than those demonstrated in our study. A possible explanation for that is the global increase of acquired antimicrobial resistance among bacteria [22,23].

In our study we found a high correlation between microorganisms found in bile and those isolated from stool. This finding, and identifying sphincterotomy as a risk factor for bile colonization, confirm the results of former studies that identify the intestinum as a starting point for ascending colonization of bile and thus for septic complications after ERCP such as cholangitis and sepsis [15–17]. The spectrum of the intestinal flora is based on a continuous interplay between the immune system and microorganisms [24]. Through the influence of immunosuppression after LT, this balance between immune system and gut flora may be disrupted, which can lead to an increased tolerance of gram-positive bacteria, especially *enterococci*, by the impaired immune system of the gut.

Interestingly, in all patients who developed signs of septicemia with the evidence of positive blood cultures, there was a match between isolates from blood and those from bile.

Conclusions

In conclusion, bile of liver transplant recipients is predominantly colonized with microorganisms. This colonization is most likely a consequence of ascension of the microorganisms from the gut. Therefore, the monitoring of bile during endoscopic intervention in the biliary tract of liver transplant patients could help to select effective prophylactic antibiotic regimes as well as to facilitate the choice of suitable antimicrobial therapy in case of cholangitis and sepsis. Gram-positive bacteria, especially *Enterococci,* seem to be frequent in bile of liver transplant recipients. Therefore, in case of septic complications after ERCP, empiric antibiotic therapy should include vancomycin or linezolid. Further prospective, large-scale, randomized trials are warranted to confirm our findings and to modulate the generally recommended antibiotic prophylaxis in case of needed endoscopic biliary interventions after LT.

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