Original paper

Microbial profile of biliary tract infection in patients undergoing therapeutic endoscopic retrograde cholangiopancreatography (ERCP), and baseline risk factors predicting microbial growth and post-ERCP cholangitis

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Abstract

Introduction: Stasis of bile flow can result in microbial colonization of the biliary tree. Cholangitis is a common adverse event linked to endoscopic retrograde cholangiopancreatography (ERCP).

Aim: To establish the bacterial profiles isolated from the bile sample and to evaluate the pre-ERCP risk factors predicting the microbial growth and development of post-ERCP cholangitis (PEC).

Material and methods: This was a prospective cohort study, which was conducted at the Department of Hepato-gastroenterology, SIUT from 1 January 2021 to 31 December 2021. Patients of either gender undergoing index ERCP procedure were included in the study. All the patients underwent ERCP, and bile culture (BC) aspirated immediately after cannulation was achieved prior to the contrast injection. There were 2 outcome variables. One was the presence or absence of organisms in bile culture, and the second one was the development of PEC.

Results: The total number of patients was 280. Bile culture was positive in 195 (69.6%) patients, and post-ERCP cholangitis developed in 187 (66.8%) patients. The most common organism in BC was *Escherichia coli* (*E. coli*), in 82 (42%) patients. History of jaundice, abdominal pain, and weight loss on admission along with ERCP performed for common bile duct (CBD) stricture were independent predictors of positive BC and PEC, while advanced age was an additional risk factor for PEC.

Conclusions: Microbial profile and risk factors for positive BC and PEC were evaluated. Advanced age, pre-operative jaundice, and prolonged biliary stasis are the independent risk factors for these conditions.

Introduction

The diagnosis and treatment of many pancreaticobiliary disorders requires endoscopic retrograde cholangiopancreatography (ERCP). The sterility of the bile duct is established by the flushing action of bile and the bacteriostatic effects of bile salts [1]. Stasis or obstruction of bile flow can result in bacterial colonization of the biliary tree [2]. The most common organisms seen in biliary tract infection are *Escherichia coli*, *Klebsiella*, and *Enterococcus* species [3].

Studies have revealed that 0.5% to 3% of the cases undergoing ERCP developed cholangitis after ERCP [4, 5]. Factors predisposing to the development of post-ERCP cholangitis (PEC) include severe nature of obstruction, tight stricture needing long duration of ERCP, lack of sterile technique, incomplete drainage, and amount of dye injected into an obstructed system [6].

PEC not only increases the length of stay in hospital and hence financial burden on the patients, but also increases the morbidity and mortality. Complications of PEC include development of sepsis, cholangitic liver

abscess, and acute renal injury. The reported mortality due to cholangitis is 4.5% [6].

Because the development of PEC carries important clinical implications, it is essential to identify factors that can predict its development, enabling better management of patients with fewer complications.

Aim

The aim of this study was to establish the bacterial profiles isolated from the bile sample and their role in selecting pre-emptive antibiotic therapy. In addition, we evaluated the pre-ERCP risk factors predicting the microbial growth and development of PEC.

Material and methods

Operational definition

Acute cholangitis [7]: Acute cholangitis was diagnosed when at least 3 of the following were present within 24–36 h of ERCP.

Clinical parameters: New-onset right upper abdominal pain.

Laboratory parameters: Rise in temperature > 38°C/100.4°F, rise in blood cells < 4 or > 10 ×/µl, rise in total bilirubin > 2 mg/dl.

Methodology

Study design: prospective cohort study.

Duration of study: January 2021 to December 2021 (1 year).

Study setting and population: Department of Hepato-gastroenterology, Sindh Institute of Urology and Transplantation.

Inclusion criteria

Patients of either gender aged > 18 years undergoing index ERCP procedure for various biliary or pancreatic disorders.

Exclusion criteria

1. Those having concurrent sepsis.
2. Failed bile aspiration.
3. Patients who had altered biliary anatomy due to previous hepatobiliary surgery, e.g. hepaticojejunostomy, Whipple's procedure, etc.
4. Patients who had previous history of ERCP.

Data collection procedure

All the patients fulfilling the inclusion criteria were enrolled in this study. After taking informed consent, the patients' demographic and clinical information were obtained and were entered into a predesigned form including the patient's gender and age, endoscopic diagnoses, preoperative jaundice, drug therapy, common bile duct diameter, and papilla types.

The ERCP interventions were performed using a therapeutic duodenoscope (TJF-260V; Olympus Optical, Tokyo, Japan). All duodenoscopes were disinfected and decontaminated according to the guidelines. The selective cannulation was performed via the common bile duct by using a guidewire in all the patients. Once the guidewire cannulation was established, bile was aspirated by inserting a single-use, 5F, standard sphincterotome catheter into the bile duct before the injection of a contrast agent for the ERCP procedure. Approximately 2–8 ml of bile (average 4 ml) was collected in a 10 ml sterile syringe.

Statistical analysis

Data entry and analysis was done using Statistical Program for Social Sciences (SPSS) version 20 (IBM Corporation, Armonk, NY, USA). Continuous variables were expressed as mean and standard deviation, while categorical variables like gender, indication of procedure, presence of Gram-positive and Gram-negative organisms and multi-drug-resistant (MDR) organisms in bile culture along with duration of treatment were presented as frequencies and percentages. There were 2 outcome variables. One was the presence or absence of organisms in bile culture and the second one was the development of PEC. A p-value < 0.05 was considered significant.

Results

The total number of patients was 280. Baseline characteristics are shown in Table I. Out of them, 145 (51.8%) patients were males. Mean age was 47.14 ±12.8 years and 154 (55.4%) patients were more than 45 years old. The most common presenting complaint was abdominal pain, which was noticed in 212 (75.7%) patients, followed by jaundice in 204 (72.9%) patients and weight loss in 143 (51.2%) patients. The most common indication for ERCP was common bile duct (CBD) stricture, seen in 164 (58%) patients, followed by CBD stone in 60 (21%) patients and chronic pancreatitis in 26 (9%) patients (Figure 1). Bile Culture was positive in 195 (69.6%) patients and post-ERCP cholangitis developed in 187 (66.8%) patients. The most common organism in bile culture was *Escherichia coli* (*E. coli*) seen in 82 (42%) followed by *Pseudomonas aeruginosa* in 50 (25.6%) patients, multiple organisms in 25 (12.8%), *Klebsiella* in 24 (12.3%), *Acinetobacter* in 12 (6.1%), and Enterococcus in 2 (1%) patients (Table II).

Out of 195 positive bile cultures, MDR organisms were noted in 119 (61%) patients. The most common

Table I. Baseline characteristics and indications of ERCP in the studied population (n = 280)

Study population		*N* (%) or mean ± SD
Age [years]		47.14 ±12.8
Age stratification	< 45 years	126 (45)
	≥ 45 years	154 (55)
Gender	Male	145 (51.8)
	Female	135 (48.2)
Indication for ERCP:		
CBD stricture		164 (58)
CBD stone		60 (21)
Chronic pancreatitis		26 (9)
Biliary leak		20 (8)
Cystobiliary communication		10 (4)
Presenting complaints:		
Jaundice		204 (72.9)
Abdominal pain		212 (75.7)
Weight loss		143 (51.2)
Bile culture:	Positive	195 (69.6)
	Negative	80 (31.4)
Pan sensitive organisms		75 (39)
Multi drug-resistant organisms		120 (61)
Post-ERCP cholangitis:	Yes	187 (66.8)
	No	93 (33.2)

ERCP – endoscopic retrograde cholangiopancreatography.

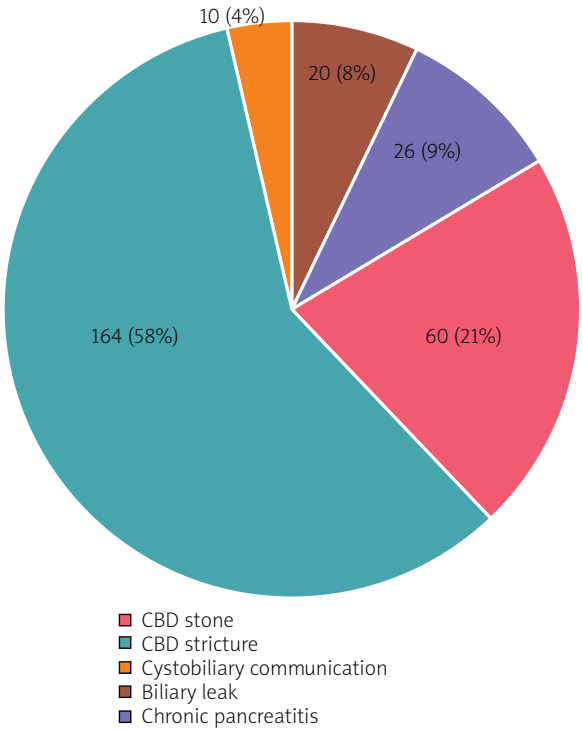

Figure 1. Indication of ERCP in the studied population

cause of MDR infection was *E. coli,* seen in 43 (36.1%) patients, followed by *Pseudomonas aeruginosa* in 25 (21%), *Klebsiella* in 20 (16.8%), multiple organisms in 20 (16.8%), and *Acinetobacter* in 11 (9%) patients (Figure 2). Most commonly, organisms were sensitive to carbapenems followed by piperacillin/tazobactam, tigecycline, cefepime, ceftriaxone, ampicillin, amoxicillin, and ciprofloxacin, respectively.

Among the 195 patients with positive bile culture, 179 developed post-ERCP cholangitis. On multivariate analysis, preoperative jaundice, history of abdominal pain, and weight loss on admission along with ERCP performed for CBD stricture and CBD stone were independent predictors of positive bile culture on ERCP (Tables III and IV), while age greater than 45 years, presence of preoperative jaundice, abdominal pain and weight loss, positive bile culture along with ERCP performed for CBD stricture were independent risk factors for the development of post-ERCP cholangitis (Tables V and VI).

Discussion

The sterility and continuous bile flow in the biliary system is an unfavourable medium for bacterial or organism growth. Blockage of bile duct due to any aetiology can result in bile stasis, allowing the bacteria and other organisms that are transferred through duodenal papillae to reside, replicate, and colonize in the bile duct, resulting in severe consequences including cholangitis and biliary tract infection.[8]

Herein, we identified the microbial profile in the patients undergoing index ERCP. The bile culture positivity rate was 74.6%, i.e. 209 out of 279 patients had positive bile culture. Gram-negative bacteria was the leading cause of bacterial growth, accounting for 86% of the positive cultures. Among Gram-negative bacteria, *E.coli* was the leading cause, followed by *Pseudomonas aeruginosa,* multiple organsims, *Acinobacter, Klebseilla,* and *Enterococci* species. Our microbial profile was comparable to that seen in other studies, and similar to intestinal flora [8–11]. A study done by Hadi *et al.* [12] reported 36% positivity of bile culture in patients undergoing cholecystectomy with Gram-negative bacteria, accounting for 80% of the positive cultures, with a micro-

Table II. Microbial profile of bile culture (n = 195)

Organism		*N* (%)
E. coli	Pan-sensitive	39 (20)
	MDR	43 (22.1)
Pseudomonas	Pan-sensitive	25 (12.8)
	MDR	25 (12.8)
Klebsiella	Pan-sensitive	4 (2)
	MDR	20 (10.3)
Acinetobacter	Pan-sensitive	1 (0.5)
	MDR	11 (5.6)
Enterococcus	Pan-sensitive	2 (1)
	MDR	0 (0)
Multiple organisms	Pan-sensitive	5 (2.6)
	MDR	20 (10.3)

MDR – multidrug resistant.

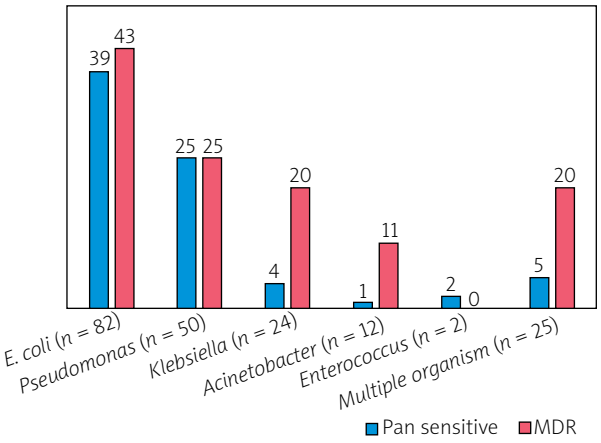

Figure 2. Microbial profile of the bile culture in the studied population

Table III. Univariate analysis for risk factors for positive bile culture

Variable		Positive culture	Negative culture	*P*-value
Age [years] mean ± SD		47.8 ±12	45 ±15	0.17
Age stratification	< 45 years	92	34	0.64
	≥ 45 years	117	37	
Gender	Male	107	38	0.80
	Female	102	33	
Indication for ERCP:				
CBD stricture	Present	150	14	≤ 0.001
	Absent	59	57	
CBD stone	Present	38	22	0.02
	Absent	177	49	
Chronic pancreatitis	Present	20	6	0.78
	Absent	189	65	
Biliary leak	Present	19	1	0.03
	Absent	189	71	
Cystobiliary communication	Present	7	3	0.73
	Absent	202	68	
Presenting complaints:				
Preoperative jaundice	Present	193	11	≤ 0.001
	Absent	16	60	
Abdominal pain	Present	183	29	≤ 0.001
	Absent	26	42	
Weight loss	Present	131	12	≤ 0.001
	Absent	78	59	
Laboratory investigations:				
Bilirubin on admission		11.1 ±4.6	9.4 ±3.3	0.006
ALT on admission		41.5 ±29.2	38 ±40.3	0.424
AST on admission		43 ±75.6	40 ±51.7	0.787
TLC on admission		10.8 ±3.3	10.8 ±2.7	0.766

CBD – common bile duct, ALT – alanine transaminase, AST – aspartate transaminase, TLC – total leucocyte count.

Table IV. Multivariate analysis for risk factors for positive bile culture

Variables	*P*-value	Odds ratio	CI (95%)	
			Lower limit	Upper limit
Presence of jaundice on admission	≤ 0.001	0.18	0.006	0.054
Abdominal pain	0.002	0.063	0.063	0.535
Weight loss	≤ 0.001	0.098	0.027	0.357
Total bilirubin > 5 mg/dl on admission	0.141	0.925	0.833	1.026
CBD stricture	0.035	0.266	0.078	0.909
CBD stone	0.04	0.245	0.061	0.983

CBD – common bile duct.

Table V. Univariate analysis for risk factors for post-ERCP cholangitis

Variable		Post-ERCP cholangitis		*P*-value
		Present	Absent	
Age [years] mean ± SD		47.8 ±12	45.3 ±14	0.171
Age stratification	< 45 years	77	49	0.034
	≥ 45 years	110	44	
Gender	Male	96	49	0.348
	Female	91	44	
Indication for ERCP:				
CBD stricture	Present	126	38	≤ 0.001
	Absent	61	55	
CBD stone	Present	39	21	0.74
	Absent	148	72	
Chronic pancreatitis	Present	16	10	0.511
	Absent	171	83	
Biliary leak	Present	14	6	0.99
	Absent	173	87	
Cystobiliary communication	Present	6	4	0.643
	Absent	181	89	
Presenting complaints:				
Preoperative jaundice	Present	171	33	≤ 0.001
	Absent	16	60	
Abdominal pain	Present	166	46	≤ 0.001
	Absent	21	47	
Weight loss	Present	115	28	≤ 0.001
	Absent	72	65	
Post-ERCP fever	Present	112	18	≤ 0.001
	Absent	75	75	
Bile culture	Positive	178	17	≤ 0.001
	Negative	11	74	
Organism on bile culture	Single	154	30	0.104
	Multiple	65	16	
MDR	Present	106	14	0.115
	Absent	65	26	
Multi drug-resistant organism:				
Escherichia coli	Present	32	7	0.814
	Absent	36	37	
Klebsiella	Present	18	2	0.05
	Absent	2	2	
Acinetobacter	Present	10	1	0.753
	Absent	1	0	
Pseudomonas	Present	22	3	0.269
	Absent	19	6	
Multiple organisms	Present	19	1	0.610
	Absent	5	0	
Difficult cannulation	Present	71	42	0.248
	Absent	116	51	
Papillotomy	Present	132	60	0.303
	Absent	55	33	
Sphinc-teroplasty	Present	39	28	0.078
	Absent	148	65	
Laboratory investigations:				
Bilirubin on admission		11.1 ±4.6	9.4 ±3.3	0.006
Bilirubin at 12 h post ERCP		10.1 ±4.1	8.4 ±3.2	0.002
Bilirubin at 24 h post ERCP		9.1 ±3.9	7.4 ±3.1	0.001
Bilirubin at 48 h post ERCP		7.9 ±3.8	6.4 ±3.1	0.004
ALT on admission		29 ±2	40 ±4.7	0.424
ALT at 12 h post ERCP		43 ±53	36 ±39	0.298
ALT at 24 h post ERCP		39 ±39	34 ±34	0.32
ALT at 48 h post ERCP		37 ±30	30 ±24	0.124
AST on admission		43 ±75.6	40 ±51.7	0.787
AST at 12 h post ERCP		48 ±131	38 ±48	0.583
AST at 24 h post ERCP		40 ±62	23 ±23	0.351
AST at 48 h post ERCP		38 ±51	32 ±21	0.264
TLC on admission		10.8 ±3.2	10.9 ±2.7	0.766
TLC at 12 h post ERCP		11.5 ±3.4	11.9 ±2.4	0.406
TLC at 24 h post ERCP		12.5 ±3.9	12.6 ±2.8	0.619
TLC at 48 h post ERCP		12.6 ±3.5	12.6 ±3.8	0.836

CBD – common bile duct, ERCP – endoscopic retrograde cholangiopancreatography, MDR – multi drug resistant, ALT – alanine transaminase, AST – aspartate transaminase, TLC – total leucocyte count.

bial profile similar to that of our population. Similarly, Ruan *et al.* reported a bile culture positivity rate of 38% in patients undergoing ERCP in a Chinese population [11]. In comparison to other studies, the high positivity of bile culture in our population was due to prolonged stasis of bile prior to ERCP, because the most common indication for ERCP in our patients was CBD stricture followed by CBD stone. There are other studies reporting variable bile culture positivity rates in different populations, ranging from 16% to 85% [9, 13–18].

Table VI. Multivariate analysis for risk factors predictive of post-ERCP cholangitis

Variables	*P*-value	Odds ratio	CI (95%)	
			Lower limit	Upper limit
Presence of jaundice on admission	0.014	2.991	0.991	9.03
Abdominal pain	0.031	0.352	0.137	0.907
Weight loss	0.049	0.409	0.168	0.996
Total bilirubin > 5 mg/dl on admission	0.305	1.08	0.929	1.266
CBD stricture	0.048	0.266	0.078	0.909
Age > 45 years	0.038	0.245	0.061	0.983
Klebsiella MDR	0.603	0.65	0.134	3.23
Bile culture	≤ 0.001	0.076	0.023	0.254

CBD – common bile duct, MDR – multi drug resistant.

In our study, bile was colonized by a single organism in most of the cases, as compared to the multiple organisms. This finding was comparable with the other studies – Ruan *et al*. and Kaya *et al.* revealed similar results [9, 11]. In contrast, a few other studies reported higher rates of multimicrobial growth in bile culture [14, 19]. This difference might be due to prolonged and overuse of antibiotics prophylactically prior to the ERCP in some areas or due to substandard culture medium.

E. coli was the most common strain colonizing the biliary tract, followed by *Pseudomonas, Acinetobacter, Klebseilla*, and *Enterococci. E. coli* is a common organism colonizing the gastrointestinal tract, while *Klebseilla* and *Acinetobacter* colonize both the gastrointestinal and respiratory tracts causing opportunistic infections [20, 21]. MDR infections were more common in our population due to easy availability of the broad spectrum antibiotics, which are prophylactically prescribed by local general practitioners. The recommended prophylactic treatment option for the prevention of PEC in patients undergoing ERCP includes cephalosporin or β-lactamase antibiotics [22]. Considering the prevalence of MDR infections in our population, most organisms were sensitive to carbapenems, followed by β-lactamase antibiotics and cephalosporins. The knowledge of this microbial profile and their antibiotic sensitivities will aid us in the usage of appropriate antibiotics for these organisms prophylactically and can also help in avoidance of antibiotic resistance.

On multivariate analysis, presence of jaundice, abdominal pain, and weight loss on admission together with ERCP performed for CBD stone and stricture were independent factors predictive of positive bile culture. This higher tendency of positive bile culture in patients with prolonged CBD stricture can be explained by the fact that biliary malignancies result in cachexia and malnutrition, leading to weight loss and decreased immunity and impaired mucosal response to pathogens resulting in bile colonization of opportunistic organisms and infection. Prolonged stasis and longer disease duration, as seen in patients with either benign or malignant CBD stricture or CBD stone, also result in biliary obstruction and proximal biliary tree dilatation causing bile stasis resulting in microbial retention and growth.

In our study, we also found that patients presenting with either jaundice, abdominal pain, weight loss with or without advanced age, undergoing ERCP for CBD stricture and positive bile culture at ERCP were at high risk of developing PEC. This can again be explained by the fact that all these findings are seen in prolonged stasis due to malignant strictures resulting in cachexia, malnutrition, and impaired immunity leading to impaired mucosal barrier causing microbial colonization and PEC. A study by Mahafzah and Daradkeh showed positive correlation between bile culture positivity and advanced age [23]. In our study, similar results were noted as the patients having age greater than 45 years were at increased risk of PEC. The knowledge of these factors causing PEC can help the clinician to start the broad-spectrum antibiotics prior to ERCP after a discussion with a multidisciplinary team involving, in particular, the infectious disease team.

Currently, prophylactic antibiotic treatment prior to ERCP is not routinely recommended. However, it is required when there is a need of repeated biliary interventions to achieve adequate biliary decompressions [22, 24].

There were certain limitations to our study. Firstly, it was a single-centred study, and secondly, the sample size was small. Hence, there is a need for a multicentric study with larger sample size to recommend prophylactic antibiotics to patients who are at high risk of developing PEC.

However, the strength of our study was that it was cross-sectional and pioneering study from this part of the world that revealed the microbial profile of biliary

tract and risk factors predictive of PEC cholangitis. We followed strict protocols to perform all ERCP procedures by sterilizing all the instruments according to the international standards prior to the procedure to avoid cross-transmission between the patients.

Conclusions

The microbial profile and risk factors for positive bile culture and PEC were evaluated. Advanced age, pre-operative jaundice, abdominal pain, and weight loss along with prolonged biliary stasis were independent risk factors for these conditions. Therefore, there should be a proper preoperative management plan including a multidisciplinary approach for the commencement of prophylactic antibiotic therapy in these high-risk patients prior to ERCP.

Conflict of interest

The authors declare no conflict of interest.

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