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Microbiology of bile in extrahepatic biliary obstruction: A tropical experience

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ABSTRACT

Purpose: Bile is considered sterile, but in obstructed biliary system, growth of micro-organisms results in bacteraemia and toxaemia. We analysed bacterial profile of patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) and evaluated antibiotic resistance patterns to formulate strategy for antibiotics in patients undergoing ERCP.

Materials and methods: Patients with cholestasis who underwent ERCP were enrolled. Bile, collected aseptically, was cultured. Positive cultures were processed for isolate identification and antibiotic susceptibility.

Results: One hundred and sixty-three patients (78 females; mean age – 55.1 ± 15.8 years) were enrolled and divided into two groups: Group I ($n = 99$) were naïve and Group II ($n = 64$) had undergone ERCP and stenting previously. Positive culture was seen in 68.1% ($n = 111$) with monomicrobial growth in 74.8% ($n = 83$) and polymicrobial growth in 25.2% ($n = 28$). Culture positivity was common in Group II vis-a-vis Group I (84.4% vs. 57.5%). Poly-microbial growth was significantly more common in Group II (35.2% vs. 15.8%, $P = 0.028$). Gram-negative bacilli were the predominant organisms isolated with *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* comprising 70% of the isolates. The most sensitive antibiotics were piperacillin-tazobactam and imipenem. The sensitivity of vancomycin, against *Enterococcus* spp. was in the range of 60%–70%.

Conclusion: Cholestasis leads to bacterial colonisation in most cases, regardless of the presence of a biliary stent. Biliary stent however predisposes to a polymicrobial growth. Most of the commonly used antibiotics continue to have significant sensitivity and may be used empirically. However, previously stented patients may have a higher incidence of infection with *Enterococcus* spp. and may require specific therapy.

Introduction

Bile is considered sterile and bacteria in the biliary system are of no clinical significance under normal circumstances. However, in patients with complete or partial biliary obstruction, biliary pressures increase along with bacterial proliferation within the stagnant bile leading to translocation of bacteria or endotoxins into systemic circulation resulting in the clinical manifestations of cholangitis which spans a spectrum from a local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndrome [1]. In bile, the typical pathogens are the Gram-negative enteric aerobes such as *Escherichia coli* and *Klebsiella* spp., while *Pseudomonas aeruginosa, Bacteroides fragilis* and *Enterococcus faecalis* are less commonly cultured. Viral and fungal agents are rare [2–4]. We analysed the bacterial profile of patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) at our centre and evaluated their antibiotic resistance patterns.

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Materials and methods

Enrolment

Consecutive patients between 18 and 65 years who underwent ERCPs between March 2016 and February 2017 at a tertiary care centre in northern India were enrolled for the study. Exclusion criteria included patients with a biliary stent without evidence of recurrent cholestasis undergoing ERCP for elective stent exchange. Patients with cholangitis who had received more than one dose of antimicrobials prior to ERCP were also excluded.

Clinical cholangitis was defined as the presence of fever with cholestatic liver function tests derangements, with or without leucocytosis and/or biliary dilation on imaging studies. Patients with frank pus from the biliary tree during ERCP were also considered as having cholangitis irrespective of fever or leucocytosis. All patients received one dose of antibiotic prior to ERCP. The antibiotic was either ciprofloxacin or cefotaxime administered intravenously within 2 h of the procedure. Cefotaxime was preferred in patients who had fever or jaundice. The basic demographic and clinical data were recorded prior to ERCP. Written and informed consent for the procedure and the study was taken after counselling of the patients and their relatives.

Sterilisation of equipment

Manual pre-cleaning was done for all side viewing endoscopes (SVEs) diligently as per protocol. The SVEs were immersed in a 1% solution of tap water and disinfectant-detergent solution. After immersion the SVE's external surface, port openings and connector caps were cleaned with a bristled toothbrush and sponge. While immersed in the bacteriostatic solution, all accessible channels of the SVE were cleaned with a channel-cleaning brush (C.R. Bard, Inc., Billerica, Mass.). Air-water and suction channels were flushed by the sterile solution using a sterile syringe. Vacuum pump (GOMCO, Allied Healthcare Products, St. Louis, Mo.) was used to suction the solution through suction-accessory channel. Thereafter, clean water was suctioned through the suction-accessory channel and pushed through the air-water channel followed by leakage testing under pressure. After rinsing, the instrument was immersed in 2% glutaraldehyde or peracetic acid at 20 °C for 45 min and then rinsed with sterile water. Water was suctioned through the channels and the excess water expelled. Finally, 70% alcohol was injected trough the channels. After 1 min of exposure, the alcohol was expelled with a 20 mL syringe.

Collection of bile and culture

Standard methodology in collection of samples was employed [5]. During ERCP, a sterile 5F standard intraductal ERCP catheter was passed into the obstructed bile duct and bile was aspirated before contrast injection into a sterile syringe (at least two samples per patients). Ten millilitres of bile were aspirated and directly inoculated into the blood culture bottles (aerobic and anaerobic) under aseptic precautions. Culture bottles were incubated for at least 5 days using automated Blood Culture BacT/ALERT 3D system (BioMerieux, France) and then discarded if negative. Bottles flagging positive were sub-cultured on to blood agar and MacConkey agar and incubated aerobically at 37 °C. Initial identification was by standard biochemical tests and ABST was carried out by Kirby-Bauer disk diffusion test. Further confirmation was done by Vitek-2 Compact Automated system (BioMerieux, France). Antibiotic susceptibilities were interpreted according to CLSI guidelines for that year [6,7].

Statistical analysis

Culture results and microbial susceptibilities were compared between patients with and without biliary stents. Statistical analysis was performed with *Z*-test (when comparing proportions), Fisher's exact (for contingency table) and *t*-tests (when comparing means). A $P < 0.05$ was considered statistically significant.

Ethical clearance

Institutional ethical clearance was taken prior to the study from the ethical committee in the hospital.

Results

From March 2016 to February 2017, 163 patients (85 males, 78 females; mean age – 55.1 ± 15.8 years, range 23–65 years) underwent ERCP. The patients were divided into two groups: Group I ($n = 99$, 60.7%) were naïve and Group II ($n = 64$, 39.3%) had undergone ERCP and stent previously. The clinical profile and investigations of the patients is depicted in Table 1 and the aetiology of biliary obstruction is shown in Fig. 1.

Growth characteristics

Bile culture was done keeping all aseptic precautions. Of all the patients, 68.1% ($n = 111$) had a positive biliary culture. Monomicrobial growth (single organism) was seen in 74.8% ($n = 83$) and poly-microbial (≥2 organisms) growth in 25.2% ($n = 28$) samples. Culture positivity was more common in Group II as compared to the Group I (84.4% vs. 57.5%, $P = 0.0003$) Fig. 2. Stent in the biliary tract impacted the nature of growth. Amongst the patients with a positive growth in Group I and II, poly-microbial growth was significantly more common in Group II as compared to Group I (35.2% vs. 15.8%, $P = 0.028$) Fig. 3.

Bacteriological profile

A positive growth was obtained from 111 bile samples, of which monomicrobial growth was obtained from 83 and polymicrobial growth from 28 samples. A total of 149 bacteria were isolated. Gram-negative bacilli were the predominant organisms isolated with *E. coli* ($n = 60$), *P. aeruginosa* ($n = 30$) and *Klebsiella pneumoniae* comprising 70% of the total isolates. In the Gram-positive group, *Enterococcus* spp. ($n = 23$) was the predominant isolate with *E. faecalis* ($n = 15$, 65.2%) being more commonly isolated as compared to *Enterococcus faecium* ($n = 8$, 34.8%) and. The growth patterns of both the groups had a similar culture profile except minor differences. First, certain organisms like *E. coli* (59.3% vs. 49.1%, $P = 0.28$) and *Enterococcus* spp. (25.9% vs. 15.8%, $P = 0.19$) were more frequently seen in patients of Group II as compared to Group I but

Table 1
Clinical profile.

	Total ($n = 163$)	Group I ($n = 99$)	Group II ($n = 64$)
Age (years)	55.2 ± 15.8	53.2 ± 16.3	58.2 ± 12.3
Females, *n* (%)	70 (42.9)	41 (41.4)	39 (60.9)
Abdominal pain, *n* (%)	94 (57.7)	58 (58.6)	36 (56.3)
Jaundice, *n* (%)	75 (46.1)	45 (45.5)	30 (46.9)
Itching, *n* (%)	30 (18.4)	19 (19.2)	11 (17.2)
Clay stools, *n* (%)	26 (15.9)	16 (16.2)	10 (15.6)
Lump, *n* (%)	12 (7.4)	7 (7.1)	5 (7.8)
Fever, *n* (%)	37 (22.7)	22 (22.2)	15 (23.4)
Chills, *n* (%)	19 (11.7)	11 (11.1)	8 (12.5)
Hemoglobin (g/dl)	11.4	11.8	11.0
Leucocyte count (‘000/cmm)	8.2	8.1	9.7
Platelet (‘00000/cmm)	2.3	2.1	2.7
Bilirubin (mg/dl)	3.8	3.4	3.5
Mean AST (IU/L)	87.2	72.4	90.4
Mean ALT (IU/L)	90.2	88.4	96.4
Mean ALP (IU/L)	311.1	365.4	215.3
Mean GGT (IU/L)	194.2	315.5	134.2

AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase.

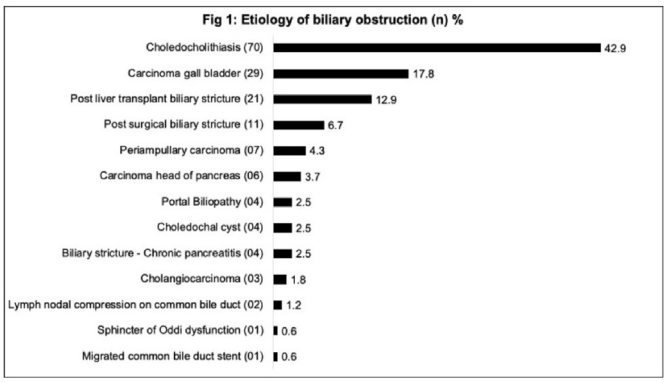

Fig. 1. Aetiology of biliary obstruction.

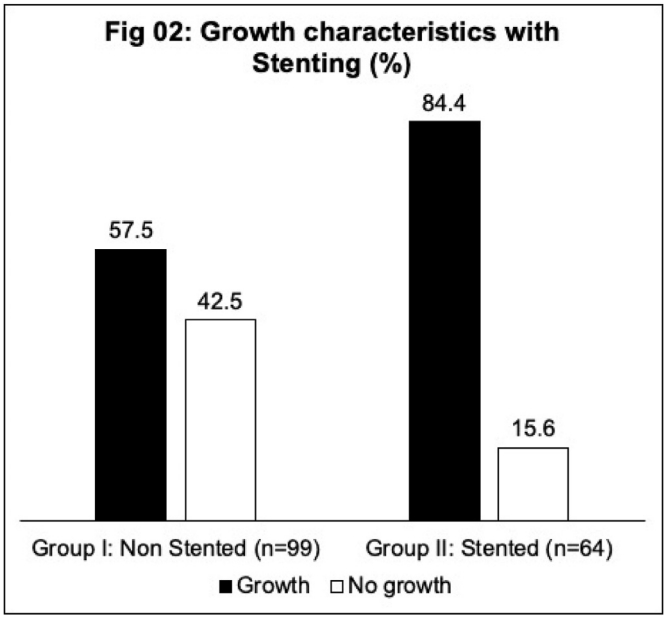

Fig. 2. Growth characteristics (growth vs. no growth) as compared to the status of stenting of the biliary system.

the difference was not significant. Secondly, the growth spectrum of stented Group II patients was more diverse with lower isolation rates of *K. pneumoniae* (9.3% vs 15.8%, $P = 0.31$) and more of *Acinetobacter* spp. (3.7% vs. 2.7%, $P = 0.76$) and other organisms like *Serratia* spp., *Enterobacter* spp., *Staphylococcus* spp. etc., (20.4% vs. 14.1%, $P = 0.38$).

Antibiotic sensitivity

The antibiotic sensitivity profile of the four most common organisms (*E. coli, P. aeruginosa, Enterococcus* spp. *and K. pneumonia*) which formed 85.2% (127/149) of the isolates were analysed. These organisms formed 87.3% (62/71) and 83.3% (65/78) isolates of Group I and II, respectively Table 2. The organism-wise antibiotic sensitivity profile is depicted in Table 3.

Most of the commonly available antibiotics were tested for antibiotic resistance. The antibiotics with most resistance were ampicillin and cotrimoxazole with resistance rates of 91.3% and 51.4%, respectively. The most sensitive antibiotics were piperacillin-tazobactam and imipenem with resistance rates of 22.1% and 25.9%, respectively. The sensitivity of vancomycin against *Enterococcus* spp. was in the range of 65%–75% Table 3 which would be desirable choice in cases of infection with these bacteria. There were some differences in the resistance patterns amongst the two groups which were either insignificant or when

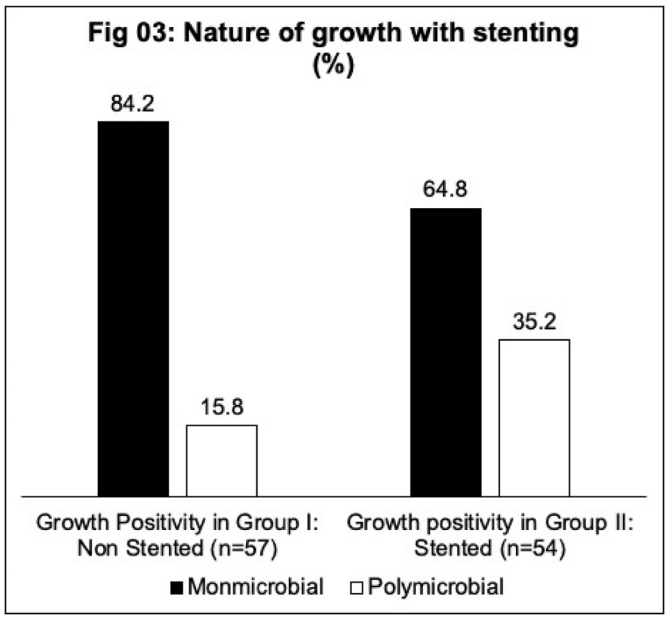

Fig. 3. Nature of growth (mono vs. polymicrobial growth) as compared to the status of stenting of the biliary system.

significant were too small to have clinical implications.

Discussion

Cholestasis due to obstruction in the biliary tree predisposes the bile to be colonised with bacteria and ultimately to cholangitis. Prophylactic use of antibiotics prior to endoscopic intervention is usually practiced before biliary cannulation [8]. The spectrum of biliary organisms and the resistance pattern helps in determining the choice of antibiotics that may be prescribed empirically. We assessed the growth pattern of organisms in bile and their resistance pattern by assessing the biliary growths in 163 patients.

Out of the cohort of patients included in our study, about 68% of them had a positive growth. Of all positively flagged blood culture bottles, most were positive in pairs (aerobic and anaerobic) with same organism being isolated from both. However, only in 9 pairs, anaerobic bottle alone flagged positive. In all these cases, facultative anaerobes were isolated. Anaerobic cultures were not performed during the study.

Although bile is a sterile medium, cholestasis predisposes to bacterial growth and biliary cultural positivity rates have been described in ranges that vary widely. Most of the older studies showed low positivity rates ranging from 26% to 48% [9,10]. However, recently conducted studies by Kaya et al. show a higher positivity rate of around 50% [5]. Gargouri et al. showed very high rates of about 93% [11]. The existing data and our study results show that the probability of bacterial growth in

Table 2
Spectrum of organisms.

	All patients ($n = 111$), n (%)	Group I (non-stented) ($n = 57$), n (%)	Group II (stented) ($n = 54$)
E. coli	60 (40.26)	28 (39.43)	32 (41.02)
Pseudomonas aeroginosa	30 (20.13)	16 (22.53)	14 (17.94)
Enterococcus spp.	23 (15.43)	9 (12.67)	14 (17.94)
Klebsiella pneumonia	14 (9.39)	9 (12.67)	5 (6.41)
A. baumannii	3 (2.01)	1 (1.4)	2 (2.56)
Others	19 (12.75)	8 (11.27)	11 (14.1)
Total	149	71	78

E. coli: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *K. pneumonia*: *Klebsiella pneumonia*, *A. baumannii*: *Acinetobacter baumannii*.

Table 3
Antibiotic sensitivity pattern (%).

	Amp	Pipt[a]	Cefta[a]	Mero[a]	Imip[a]	Amik[a]	Cipro[a]	Co-tri[c]	Tige[b]	Vanco[d]
Antibiotic sensitivity of 4 commonest organisms in all patients: Total bile samples – 163, culture +ve samples – 111, no growth – 52, growth count of 4 commonest organisms – 127										
E coli (60)	18.3	76.7	60	66.7	71.7	80	55	46.7	68.3	–
P. aeroginosa (30)	0	73.3	73.3	50.0	83.3	70	56.7	–	–	–
Enterococcus spp. (23)	0	–	–	–	–	–	–	–	–	69.6
K. pneumoniae (14)	0	92.9	50	92.9	64.3	42.9	85.7	57.1	92.9	–
Total (127)	8.7	77.9	62.5	65.4	74.1	72.1	59.6	48.6	73.1	69.6
Antibiotic sensitivity of 4 commonest organisms in Group I (non-stented): Total bile samples – 99, culture +ve samples – 57, no growth – 42, growth count of 4 commonest organisms – 62										
E coli (28)	17.9	82.1	64.3	67.9	71.4	78.6	53.6	53.6	71.4	–
P. aeroginosa (16)	0	62.5	68.8	50	81.3	75	50	–	–	–
Enterococcus spp (9)	0	–	–	–	–	–	–	–	–	77.8
K. pneumoniae (9)	0	88.9	44.4	100	44.4	33.3	66.7	55.6	88.9	–
Total (62)	8.1	77.4	62.3	67.9	69.8	69.8	54.7	54.1	75.7	77.8
Antibiotic sensitivity of 4 commonest organisms in Group II (stented): Total bile samples – 64, culture +ve samples – 54, no growth – 10, growth count of 4 commonest organisms – 65										
E. coli (32)	18.8	71.9	56.3	65.6	68.8	75	50	40.6	65.6	–
P. aeroginosa (14)	0	85.7	78.6	50	78.6	64.3	64.3	–	–	–
Enterococcus spp (14)	0	–	–	–	–	–	–	–	–	64.3
K. pneumoniae (5)	0	100	60	80	100	60	80	60	100	–
Total (65)	9.2	78.4	62.7	60.8	74.5	70.6	56.9	43.2	70.3	64.3

Amp: Ampicillin, Pipt: Piperacillin-tazobactam, Cefta: Ceftazidime, Mero: Meropenem, Imip: Imipenem, Amik: Amikacin, Cipro: Ciprofloxacin, Co-tri: Co-trimoxazole, Tige: Tigecyclin, Vanco: Vancomycin, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *K. pneumonia*: *Klebsiella pneumonia*, *A. baumannii*: *Acinetobacter baumannii*.

[a] The average sensitivity of piperacillin-tazobactum, ceftazidime, 'penems, amikacin and ciprofloxacin has been calculated with non-*Enterococcus* isolates only'.
[b] The average sensitivity of tigecycline has been calculated with non-*Enterococcus*, non-pseudomonal isolates only.
[c] The average sensitivity of cotrimoxazole has been calculated with non-*Enterococcus*/non pseudomonal isolates only.
[d] The average sensitivity of vancomycin has been calculated with *Enterococcus* isolates only

cholestatic bile is high and thus may routinely warrant antibiotics prior to biliary cannulation. As most of the bile is infected/colonised, it becomes important to know the growth and sensitivity patterns as they may vary in various regions of the world. It is with this idea that this study was carried out in a tropical country in Asia.

The most common bacteria grown in our study were *E. coli* (40.26%), *P. aeruginosa* (20.13%), *Enterococcus* spp. (15.43%) and *K. pneumoniae* (9.39%). Kaya et al. had encountered *E. coli* (28.2%) and *P. aeruginosa* (17.3%) in their study [5] and Suna et al. observed *E. coli* (32.8%), *Enterococcus* spp. (26.2%) and *P. aeruginosa* (11%) which are similar to our study [12]. Similar results have been documented by other studies over the past two decades [13] and lately Basioukas et al. showed *E. coli* in about 50% growth which is similar to our results [14].

A loss of the barrier between the biliary tract and the duodenal lumen, due to the stent placement, is an important factor in ascending bacterial ductal colonisation [15], polymicrobial cultures [14] and biliary stent associated cholangitis [16]. We demonstrated that stenting had a significant impact on the positivity of culture [with 84.4% of stented patients having a growth as compared to 57.5% of unstented patients: Fig. 2] and polymicrobial growth [35.2% in stented vs. 15.8% in unstented: Fig. 3]. Our study also showed that stenting contributes to a change in the bacterial spectrum with a higher contribution by *Enterococcus* spp. Similar results of a polymicrobial growth and *Enterococcus* spp. predominance (25%–30% of all growths) in patients with biliary endoprosthesis have been shown previously by Basioukas et al. with the most frequently isolated organisms being *Enterococcus* spp. (74%), *E. coli* (62%) and *K. pneumoniae* (58%) [14]. Cultures growing *Enterococcus* spp. in our study had two additional notable aspects. First, *Enterococcus* spp., being more common in patients with stents, helped us predict that they could be targeted in patients with existing stents. Second, most of the antibiotics targeting *Enterococcus* spp. show acceptable sensitivity and choice in such cases may not be as limited as expected.

In our study, the most sensitive antibiotics were piperacillin-tazobactam and imipenem with resistance rates of 22.1% and 25.9%, respectively. Gargouri et al. had demonstrated similar although better sensitivity pattern in their study in which Imipenem showed the best antimicrobial activity (sensitivity, 100%) [11]. Kaya et al. had similar findings in their study [5]. The antibiotics with most resistance in our study was ampicillin and cotrimoxazole with resistance rates of 91.3% and 51.4%, respectively. The sensitivity of vancomycin against *Enterococcus* spp. was in the range of 65%–75% which was lower as compared to the findings of Suna et al. [12] and Lorenz et al. [13] wherein they demonstrated had 93% sensitivity and 82%, respectively. Nevertheless, the clinical response to vancomycin in our study based on the culture sensitivity was optimal.

It is a common practice for a long time that cephalosporins and quinolones are used as antibiotic prophylaxis in patients undergoing ERCP. Most of the initial data that led to such practice was from bile of surgically resected specimen [9,10]. However, studies by Rerknimitr et al. [16] and more recent ones like Kaya et al. [5] also show excellent sensitivities of routinely prescribed antibiotics. After analysing the results in our study, a similar result was seen with commonly used antibiotics such as ceftazidime, amikacin and ciprofloxacin continuing to have overall sensitivity rates of above 50% Table 3 making them a good choice as a first agent for empirical antibiotic in ERCP. The result indicates (although cannot be extrapolated with confidence) that similar results would be applicable to other cephalosporins as well.

An interesting outcome in our study is that the overall resistance to meropenem is more than that of imipenem both in the stented and non-stented groups. These imipenem sensitive meropenem resistant isolates have been reported in other studies as well [17,18]. There are multiple mechanisms of carbapenem resistance which can be either plasmid and chromosomal mediated. In *P. aeruginosa*, studies have demonstrated over expression of efflux pumps contribute to these phenotypes [19]. However, in *Enterobacteriaceae,* the emergence of isolates with different susceptibilities to carbapenems may be attributed to mechanisms other than cabapenemases such as overproduction non-carbapenemases β-lactmases, like AmpC, along with down regulation porins in outer membrane proteins [20]. The study of mechanism of resistance could provide useful insights into the study of antibiotic resistance, however, the same was not conducted in our study and would be a limitation. In addition, we did not further culture the anaerobic growth, thus being a limitation of our study.

Most of the patients received a single dose of antibiotic prior to the procedure which is a standard practice. Most clinical guidelines

recommend single antibiotic use unless the patient has severe cholangitis. We also recommend following the guidelines. However, our study was not designed to assess the benefits of using more than one antibiotic as compared to a single antibiotic.

Conclusion

To summarise, cholestasis is associated with bacterial growth in almost two-third of cases, regardless of the presence of a biliary stent. Biliary stent, however, predisposes to a higher incidence of polymicrobial bacterial growth. Most of the commonly used antibiotics continue to have significant sensitivity and may be used empirically. Quinolones or a third-generation cephalosporin, which penetrate an obstructed biliary system, can provide excellent broad-spectrum coverage before ERCP which may be upgraded to carbapenems which have shown high sensitivity against expected pathogens, if the clinical situation and bile culture merits the same. In addition, patients who have a stent in place may benefit from a further coverage against *Enterococci* spp.

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Nil.

Declaration of competing interest

There are no conflicts of interest.

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