Obstructive jaundice: Studies on predictors of biliary infection and microbiological analysis in an HIV setting

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Background. Early diagnosis of biliary infection is critical for timely antimicrobial therapy and biliary drainage. HIV infection may influence the spectrum and severity of biliary infection in an environment with a high HIV prevalence. Charcot’s triad has low sensitivity and higher specificity for biliary infection, and more sensitive markers are required.

Methods. To investigate possible predictors of biliary infection (bacteriobilia) and identify the microbiological spectrum in patients presenting with biliary obstruction to a tertiary institute in an environment with a high prevalence of HIV.

Results: One hundred and six patients with obstructive jaundice had a mean age of 52 years (range 21 - 58); most were female (74%), and 36 (34%) were infected with HIV, with a mean CD4 count of 495 cells/µL. Cholelithiasis (53%), biliary strictures (21%) and head of pancreas tumour (8%) were the main aetiopathologies. Bile was obtained for microbial culture from 104 patients (98%), and 56 (54%) were infected with HIV, with a mean CD4 count of 495 cells/µL. Cholelithiasis (53%), biliary strictures (21%) and head of pancreas tumour (8%) were the main aetiopathologies. Bile was obtained for microbial culture from 104 patients (98%), and 56 (54%) were infected with HIV, with a mean CD4 count of 495 cells/µL. Gram-negative bacteria were most frequent (58%), and 2 HIV-infected patients had fungal infections (Candida albicans and Aspergillus fumigatus). Screening for endoscopy-associated infections revealed Pseudomonas aeruginosa. PCT was a poor predictor of bacterial infection, whereas CRP was a fair predictor.

Conclusions. The majority of bacteria cultured were sensitive to ciprofloxacin or amoxicillin-clavulanate. Duodenoscopes were a potential source of Pseudomonas infection.

The prevalence of HIV infection in South Africa (SA) was estimated at 7.52 million cases (13.1%) in 2018. Since HIV may affect the prevalence and severity of sepsis, it is pertinent to examine biliary sepsis in this setting of a high HIV infection rate.

Knowledge of the spectrum of infecting organisms in a practice location facilitates early empirical antimicrobial therapy, as the infecting organism and its sensitivity may not be apparent at initial presentation. This spectrum and sensitivity may change over time. The sources of infections in the bile are reflux of duodenal contents, blood-borne infection or the portal-venous channels, and the concentration of organisms is increased in patients with cholangitis. Csendi et al demonstrated that >10⁸ organisms were seen in 0% of normal patients, 3% of patients with symptomatic gallstones, 36% of patients with common duct stones but without cholangitis, and 85% of patients with acute cholangitis. Choledocholithiasis is the leading cause of biliary obstruction, although periampullary malignancies and benign strictures have become increasingly prevalent.

Acute cholangitis has a wide aetiological and clinical presentation, and may be fatal in the more severe forms. Bacterial contamination of the obstructed bile (bacteriobilia) is the initial event, which may then progress to suppurrative cholangitis, liver abscesses, bacteraemia and septicemia. The early diagnosis of acute cholangitis is critical for the initiation of antimicrobial therapy and biliary drainage. Charcot’s triad is used as a marker of cholangitis, and although specific, it has been demonstrated to have sensitivity as low as 22%, and 26.4% in a controlled study. Cholangitis is currently graded as I - III based on clinical, haematological and imaging criteria.

Obstructive jaundice is mainly relieved by endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography (PTC), surgical procedures and increasingly endoscopic ultrasound techniques. Non-selective relief of obstructive jaundice may result in biliary contamination and septic complications after definitive surgery for the underlying conditions. Nosocomial infection of the endoscopic equipment has also been demonstrated and linked to infections in patients. In patients with cholangitis, poor nutrition and renal failure, biliary decompression is necessary to manage these complications, but the benefits of preoperative biliary drainage in patients with asymptomatic bacteriobilia are unclear, as this condition is difficult to identify.

It has been noted that the spectrum of microbial agents causing acute cholangitis may change over time and result in resistant flora. Periodic evaluation is therefore required to ensure that antibiotic regimens remain relevant and effective.

C-reactive protein (CRP) has been utilised for predicting biliary infection. Procalcitonin (PCT) has been proposed as of more value in predicting the outcome of sepsis than in diagnosing sepsis.
Previous studies have not examined the aetiology and prediction of biliary infection against the background of an HIV-infected population, as is the case in SA. There are also no reliable markers of asymptomatic bacteriobilia, which requires invasive procedures for diagnosis. This lack was an additional motivation to investigate some of the known markers of sepsis in determining the presence of bacteriobilia in patients with obstructive jaundice.

**Objectives**

To: (i) determine the prevalence of bacteriobilia in patients presenting for therapeutic ERCP, PTC and surgery; and (ii) assess the bile microbial spectrum and antimicrobial sensitivity in patients with biliary obstruction and the association with HIV infection.

**Methods**

**Patients**

This was a prospective study conducted from June 2018 to June 2019. All patients aged ≥18 years who presented with obstructive jaundice for ERCP, PTC or surgery were included. All patients were antibiotic naive and had not previously had ERCP, PTC or surgery. Informed consent was obtained from all patients.

**Methods**

At presentation, patient demographic information and HIV status (including treatment with antiretrovirals) were obtained, clinical assessment was performed (abdominal pain, temperature, pulse, blood pressure), laboratory tests for haematological, renal and liver function were carried out, and imaging by ultrasound or computed tomography was done. All patients were offered testing for HIV infection unless they were confirmed infected, and those who declined testing were excluded from the study.

All patients were mentally orientated. At ERCP, PTC or surgery, 2 - 5 mL of bile was aspirated prior to injection of contrast with single-use biliary access devices and sterile syringes. These specimens were collected in sterile containers for microbial cultures and assessment of antimicrobial sensitivity. Antibiotic prophylaxis was not routinely used, and patients with grade II or III cholangitis were treated with ciprofloxacin empirically. The presence of biliary sepsis was defined as per the Tokyo 2018 diagnostic criteria for acute cholangitis.30 An international normalised ratio (INR) <1.30 was necessary according to the unit's protocol for invasive procedures in obstructive jaundice. Specific biomarkers assessed for predicting biliary infection were clinical/haematological criteria, CRP and PCT. ROC curves were evaluated for clinical/haematological criteria, CRP and PCT. 

**Discussion**

When compared with other studies (Table 1), the present study had more females in the cohort, but similar to the other studies, the mean age exceeded 50 years. There are inconsistencies between reported studies in the proportion of patients presenting with choledocholithiasis and malignant disease (Table 1). Cholecystolithiasis is a leading cause of biliary obstruction, and this is evident in our study (53%), but in three of the other studies malignant disease was the main cause.4,12,13 The 34% prevalence of HIV infection in the present study is much higher than the 16%
### Table 1. Comparison of demographics, aetiology, biliary procedures, spectrum of bacteria in bile and antimicrobial resistance

| Study | Sharma et al., 2016⁷ | Sahu et al., 2011⁸ | Alves et al., 2016⁹ | Kwon et al., 2013¹⁰ | Rupp et al., 2016¹¹ | Present study, 2019 |
|-------|----------------------|------------------|-------------------|---------------------|----------------------|---------------------|
| **Sex, n (%)/n** | | | | | | |
| Male | 154 (51) | 102 (55) | 18 | 903 (64.4) | ns | 28 (26) |
| Female | 147 (49) | 83 (45) | 12 | 500 (35.6) | ns | 78 (74) |
| **Age (years), mean (range/SD)** | 58 (22 - 96) | 51.3 (13.4) | 57.7 (15.6) | 60.8 (12.9) | 55.6 (15.6) | 51.56 (16.43) |
| **Patient selection – prior antibiotics, n** | ns | ns | ns | 0.2% | 1 401 (100) | 99 |
| **Procedures to collect bile, n/%/n (range/SD)** | | | | | | |
| ERCP | 208 | 133 | 0 | 81.1% | 0 | 2 |
| PTC bile duct | 93 | 15 | 0 | 8.3% | 0 | 0 |
| Surgery | 0 | 0 | 30 | 4.3% | 0 | 3 |
| T-tube | 0 | 0 | 0 | 4.3% | 0 | 0 |
| **Bile collection** | ns | 10 mL in 95 patients (51%) | ns | ns | ns | 2 - 5 mL. |
| **Bacterobilia, %/n (%)** | 78 | 47 (26) | 12 (40) | 54 | 72 | 56 (54) |
| **Aetiology, %/n (%)** | | | | | | |
| Choledocholithiasis | 30 | 116 (63) | 12 (40) | 499 (36) | 153 (11) | 56 (53) |
| Benign stricture | 0 | 17 (9) | 0 | 0 | 0 | 6 (6) |
| Malignant stricture | 51 | 52 (28) | 18 (60) | 904 (64) | 366 (26) | 0 |
| Stricture, ?aetiology | 0 | 0 | 0 | 0 | 0 | 35 (33) |
| Orthotopic liver transplantation | 0 | 0 | 0 | 0 | 427 (31) | 0 |
| PSC | 0 | 0 | 0 | 0 | 222 (16) | 0 |
| Other | ns | ns | ns | ns | 233 (17) | 9 (8) |
| **p-value** | <0.001 | | | | | |
| **Cholangitis (grades II + III), %/n (%)** | 70 | 100 | ns | 1 095 (78) | 202 (18) | 25 (24) |
| **p-value** | <0.00001 | | | | | |
| **Bacterobilia patient characteristics, %/n (%)** | 94 | 0 | ns | ns | 682 (58) | 0 |
| **Prior biliary procedure** | 60 | 100 | ns | ns | 478 (42) | 100 |
| **p-value** | <0.00001 | | | | | |
| **HIV-positive** | ns | ns | ns | ns | ns | 36 (34) |
| **HIV-negative** | ns | ns | ns | ns | ns | 65 (61) |
| **Organisms, %/n (%)** | 50 | ns | 58 | 63 | 30 | 25 (45) |
| Monomicrobial | 50 | 70 | 42 | 38 | 39 | 31 (55) |
| Polymicrobial | 17 | 38 | 22 | ns | 57 | 52 |

Continued ...
Bacteriobilia and fungobilia were identified in bile cultures of 56 patients (54%) in the present study. The prevalence of bacteriobilia ranges from 26% to 78% in other research (Table 1).

The diagnostic and grading criteria for acute cholangitis have been revised to utilise a combination of inflammatory, cholestasis and imaging criteria to arrive at a suspected or definitive diagnosis of acute cholangitis, grading it into mild, moderate and severe forms of the complication of obstructive jaundice.

Grade I (mild) cholangitis does not meet the criteria of moderate cholangitis or the presence of organ dysfunction. In grade II (moderate) cholangitis, two of the following are present: (i) white cell count count >12 or <4 × 10⁹/L; (ii) high fever (≥39°C); (iii) age ≥75 years; (iv) bilirubin ≥85.5 µmol/L; and (v) albumin <24.5 g/L. Grade III (severe) disease is associated with organ dysfunction in at least one of the following systems: (i) cardiovascular: hypotension requiring dopamine ≥5 µg/kg/min or any dose of norepinephrine; (ii) nervous: disturbance of consciousness; (iii) respiratory: partial pressure of oxygen/fraction of inspired oxygen ratio <300; (iv) renal: oliguria, serum creatinine >176.84 µmol/L; (v) hepatic: platelets (INR >1.5); and (vi) haematological: platelet count <100 × 10⁹/L. The majority of patients in the present study (76%) had grade I cholangitis (Table 3). In three of the studies in Table 1, the majority of the patients had clinical cholangitis (70 - 100%), and in one they were in the minority (18%). In the study by Sharma et al., bacteriobilia was more frequent in patients with clinical cholangitis than in those without (91% vs. 58%). This differs from the findings of the present study, where the figures for the two groups were 40% and 54%, respectively.

Although HIV was associated with a higher prevalence of infection, 58% (n=21/36) v. 51% (n=33/65) in HIV-negative patients (p=0.8), the bacterial content in HIV-infected patients was similar to that in the non-HIV-infected patients, and HIV-infected patients comprised the group with fungal infections (Table 2).

Gram-negative bacilli were the dominant infecting organisms in the present study and three of the other studies (Table 1). Enterococcus was found to be the dominant organism in a Korean study and in a German study. The frequent findings of streptococcal strains suggest contamination from oral flora, but these were not isolated in the endoscope washings and should be evaluated further with more frequent culture of endoscope washings.

Bacterial sensitivities varied across studies, and in the present study, the Gram-negative bacilli were sensitive to the quinolones and amoxicillin-clavulanate in 86 (100%) of cultures and in all but one (Staphylococcus – vancomycin) of the Gram-positive bacilli. This sensitivity profile is different to that demonstrated by Sharma et al., where Gram-negative bacilli were more sensitive to carbapenems.
(51 - 100% sensitivity) and piperacillin-tazobactam (28 - 88% sensitivity), but there was a high frequency of resistance to third-generation cephalosporins (5 - 37% sensitivity) and quinolones (19 - 59% sensitivity). These authors also demonstrated that Gram-positive cocci were less sensitive to quinolones (29 - 62% sensitivity) and more sensitive to vancomycin (69 - 100% sensitivity) and amoxicillin (46 - 76% sensitivity). These findings are similar to those of Sahu et al.,[15] who demonstrated high resistance to the quinolones (14 - 50% sensitivity) and third-generation cephalosporins (20 - 50% sensitivity) among Gram-negative bacilli. They also found high resistance to the quinolones (26% sensitivity) among the Gram-positive bacilli, but there was susceptibility to vancomycin (95% sensitivity). In the German study,[13] there was much less resistance to the quinolones, third-generation cephalosporins, piperacillin-tazobactam and the carbapenems, with sensitivities of 75%, 89%, 87% and 99%, respectively. The figures for the Gram-positive bacteria were 46% and 60% for the quinolones and piperacillin-tazobactam, and 59% and 89% for the carbapenems and vancomycin.

PCT is now widely utilised in the diagnosis of bacterial infection and determining the response to antimicrobial therapy. In the present study, PCT was found to have a low sensitivity (76%) and specificity (66%) and an AUC of 0.58 for diagnosing bacteriobilia when all patients were assessed, which emphasises that it has value in patients who have systemic signs of infection and not localised infection. There was also no difference between the CRP and PCT values in grade I and II cholangitis, and although the values were significantly higher in grade III cholangitis, they were unable to differentiate between those with and without bacteriobilia (3.9% v. 4.5%, PCT >10 ng/mL).

If the present biochemical and clinical markers are poor predictors of bacteriobilia, are there other possibilities? In the study by Sahu et al.,[15] bacteriobilia was found in 12 of 30 bile samples (40%), being more prevalent in benign disease (n=8), in patients >50 years old (n=1) and in females (n=10).[15] In a study by Alves et al.[16] of 30 bile samples,
of which 12 (40%) were infected, bacteriobilia was associated with benign biliary pancreatitis (67%; p=0.02), female sex (83%; p=0.058) and age >50 years (92%; p=0.20), without significant associations with comorbidities (p=0.654) or gallstones (p=0.073). In the study by Sharma et al.,[12] of 505 bile samples, of which 396 (78%) were infected, bacteriobilia was more frequent (85% v. 73%; p=0.001) in benign disease. In the present study, age (p=0.208) and bilirubin (p=0.38) had no significant influence on the presence of biliary infection.

Conclusions
Bacteriobilia in HIV-positive patients does not differ from that in the general HIV-negative population. Gram-negative bacilli remain the dominant infecting organisms, and the majority are sensitive to ciprofloxacin or amoxicillin-clavulanate and are appropriate for empirical therapy. Duodenoscopes are a potential source of Pseudomonas infection and should be periodically screened and quarantined. The majority of screened patients had grade I cholangitis.

Declaration
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Author contributions
KSC, FA, FM: data collection and final manuscript. BT: statistics and final manuscript.

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Conflicts of interest
None.

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