

Profile and predictors of bile infection in patients undergoing laparoscopic cholecystectomy

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ABSTRACT

الأهداف: دراسة الشاكلة الجرثومية للصفراء وتحديد منبئات إنتان الصفراء والمضاعفات الإنتانية بعد عملية استئصال المرارة بالمنظار.

الطريقة: تم في هذه الدراسة المقطعية مراجعة 1248 حالة لعملية استئصال مرارة بالمنظار أجريت خلال الفترة ما بين عامي 1994 و 2007 من قبل فريق جراحي واحد في مستشفى الجامعة الأردنية. وتمت زراعة الصفراء لكل مريض ودرست نتائج الزراعة في ضوء مضاعفات ما بعد الجراحة. تمت دراسة منبئات ممكنة عن إنتان الصفراء بما فيها العمر والجنس والأمراض المصاحبة ومضاعفات ما بعد الجراحة والتصوير الشعاعي المراري البنكرياسي التنظيري الراجع ما قبل الجراحة (ERCP) ودواعي الجراحة.

النتائج: تم تشخيص حصى المرارة غير المتصاحبة بمضاعفات لدى 993 (79.6%) مريض بينما عانى 221 (17.7%) مريض من التهاب المرارة الحاد وعانى 34 (2.7%) مريض من أعراض اليرقان وقد خضع 132 (10.6%) من المرضى للتصوير الشعاعي المراري البنكرياسي التنظيري الراجع ما قبل الجراحة وشخصت أمراض مصاحبة لدى 513 (41.1%) مريض وظهرت مضاعفات إنتانية بعد الجراحة لدى 25 (2%) مريض. كانت الزراعة إيجابية لدى 250 (20%) مريض حيث عزلت جراثيم سالبة تفاعل الغرام من 134 (53.6%) من الحالات الإيجابية وجراثيم ايجابية تفاعل الغرام من 73 (29.2%) من الحالات الإيجابية في حين كانت النتائج مختلطة لدى 43 (17.2%) من الحالات. بين فحص مربع كأي أن العمر والجنس والتصوير الشعاعي المراري البنكرياسي التنظيري الراجع ما قبل الجراحة ووجود أمراض مصاحبة وداء المرارة المصحوب بمضاعفات هي منبئات ذات دلالة لأنتانات الصفراء في حين بين التحليل متعدد الحدود التقهقري أن العمر والتصوير الشعاعي المراري البنكرياسي التنظيري الراجع ما قبل الجراحة فقط هما منبئات مهمان لإنتان الصفراء.

خاتمة: إنتان الصفراء مضاعفة شائعة لداء حصى المرارة قد يتأثر بالعمر والمداخلات التنظيرية ما قبل الجراحية ولكنه لا يؤثر في حدوث المضاعفات الإنتانية ما بعد الجراحية.

Objectives: To study the bacteriological profile, and to determine predictors of bile infection and septic complications following laparoscopic cholecystectomy.

Methods: This cross-sectional study reviewed 1248 laparoscopic cholecystectomy cases performed between January 1994 and December 2007 by one surgical team at the Jordan University Hospital, Amman, Jordan. Bile cultures were performed for all patients and statistical analysis was performed on culture results and postoperative complications as well as, on the possible predictors of bile infection including age, gender, associated diseases, preoperative retrograde cholangiopancreatography (ERCP), and indications for surgery.

Results: Uncomplicated gallstone disease was diagnosed in 993 patients (79.6%), 221 patients (17.7%) had acute cholecystitis, and 34 patients (2.7%) had jaundice. Associated morbidities were present in 513 patients (41.1%), preoperative ERCP was performed for 132 patients (10.6%), and postoperative septic complications developed in 25 patients (2%). Bile culture was positive in 250 patients (20%), 134 (53.6%) of whom had Gram negative bacteria, 73 (29.2%) had Gram positive bacteria, and 43 (17.2%) had mixed cultures. The chi-square test has shown that positive bile culture is significantly associated with age, gender, preoperative ERCP, associated morbidities, and complicated gallbladder disease, whereas multinomial regression analysis has shown that age and preoperative ERCP were the only significant predictors of bile infection.

Conclusion: Bile infection commonly complicates gallstone disease, and it can be influenced by age and preoperative endoscopic interventions, but it does not influence the occurrence of postoperative septic complications.

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Gallstone disease is a common condition that affects millions of people worldwide, and is the most common indication for gallbladder surgery, although it is asymptomatic in most patients.^{1,2} Laparoscopic cholecystectomy (LC), a safe and effective procedure,^{3,4} has become the treatment of choice for symptomatic gallstone disease.^{2,5} Bile infection was documented in association with cholelithiasis, as well as other conditions, especially common bile duct disease.^{6,7} The frequency of bile infection in association with gallbladder disease varies greatly.^{8,9} Factors that increase the risk of bile infection includes advanced age, diabetes mellitus, hyperbilirubinemia, acute cholecystitis, use of invasive procedures, and cholesterol stones.^{10,11} Most studies have shown that Gram negative *bacilli* are the most common cause of bile infection.^{12,13} Postoperative septic complications occur at a lesser rate in elective laparoscopy, compared to open cholecystectomy.^{4,14} The correlation between positive bile culture and postoperative infectious complications is still controversial. This is probably influenced by preoperative antibiotic prophylaxis, which is advocated either for high risk groups,¹⁵ or routinely for all patients regardless of risk factors,^{16,17} whether gallstone disease is acute or chronic.¹⁸ Factors that were incriminated to increase the rate of wound infection following cholecystectomy are the same as those that increase the frequency of bile infection. Additional risk factors however, includes bactobilia, the emergency of operation, hypertension, fever on admission, and so forth.^{12,19} The aim of this study is to review the bacteriological profile of gallbladder bile cultures in a large series of LCs performed at the Jordan University Hospital (JUH), and to identify predictors of positive bile cultures and postoperative septic complications.

Methods. Patients and surgical procedures. This is a retrospective study in which 1248 laparoscopic cholecystectomies performed by one surgical team were reviewed. The study was carried out at the Jordan University Hospital, Amman, Jordan, a 520-bed tertiary care referral center in the period between January 1994 and December 2007. The study was approved by the Ethics Research Committee of the Jordan University Hospital. Indications for surgery were gallstone disease and its complications. Patients ranged in age from 5-94 years (mean \pm standard deviation = 47.2 \pm 15.22). The majority of patients (936) were women, whereas the number of men included in this study was 312 (25%), with a male to female ratio of 1:3. Upon admission to the hospital, each patient was evaluated utilizing history and physical examination, and laboratory investigations that included a complete blood count, liver and kidney functions, and other investigations depending on the health status of the patient. Patients with jaundice,

acute pancreatitis, cholangitis, or common bile duct (CBD) dilatation to >8 mm on ultrasonography, with abnormal liver functions, underwent endoscopic retrograde cholangiopancreatography (ERCP) as described previously,²⁰ to exclude concomitant CBD stones. The same surgical technique was used to perform LC for all patients, and those who were converted to open surgery were excluded from the study. Patients with non-complicated gallstone disease received a single dose of a second generation cephalosporin within one hour of surgery, whereas those with acute cholecystitis received 3 doses of the same antibiotic, where the first dose was given within one hour of surgery, and the other 2 doses were given postoperatively. Patients were followed up for a minimum of 6 months.

Bile culture. The specimen was obtained from the gallbladder bile of each patient upon removal of the gallbladder by a member of the surgical team using a sterile culture swab that was immersed in transport media, and sent to the microbiology laboratory for processing. Swabs were cultured directly onto blood agar and cystine lactose electrolyte deficient (CLED) agar plates, and were then placed in culture tubes containing thioglycollate broth, and incubated with inoculated plates for 24 hours at 37°C, after which period, they were subcultured on the same media and incubated overnight at 37°C. Microorganisms growing from direct cultures, or from subcultures were identified to the species level on the basis of colonial morphology, and by using standard microbiological techniques and tests including gram-staining, biochemical profile, and serotyping. Commercially available biochemical test panels (API, BioMerieux, Marcy-L'Etoile, France, or Remel, Lenexa, Kansas, USA) were used to identify isolates. Pure cultures were tested for antimicrobial susceptibility using the disc diffusion method. Different panels of antimicrobials were used depending on the type of the organism isolated, and results were reported as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.²¹

Statistical analysis. Descriptive statistics were applied on the results of bile cultures, which were grouped into 4 categories: negative cultures, Gram-negative bacteria, Gram-positive bacteria, and mixed cultures. The chi-square test and multinomial logistic regression analysis were applied in this study. The chi-square test was used to determine the significance between bile culture results as a dependent variable, and many independent variables that includes age, gender, presence of associated morbidities, complications, the performance of ERCP, and the indication for surgery (elective non-complicated gallstone disease, acute cholecystitis, and acute cholangitis/acute biliary pancreatitis). Multinomial

logistic regression analysis between the different categories of bile culture results (dependent variable), and the independent variables mentioned earlier were used to determine the predictors of bile infection. The Statistical Package for Social Sciences software (SPSS Inc., Chicago, IL., USA) version 9 for Microsoft was utilized, and a *p*-value ≤ 0.05 was considered statistically significant.

Results. Clinical findings. The indications for surgery such as cholelithiasis (the most common), acute cholecystitis, and jaundice with the clinical findings such as associated morbidities, the most common of which was diabetes mellitus, obesity, and cardiovascular disease, preoperative ERCP, and post-operative septic complications of surgery in the form of surgical site infection and peritonitis are shown in Table 1.

Bacteriological profile of bile cultures. Culture results of gallbladder bile are summarized in Table 2. Positive cultures were detected in 250 (20%) specimens, more than half of which (53.6%) grew Gram negative bacilli (Table 2). The majority of isolates (77%) recovered in this study belonged to 6 genera (Table 3). These includes *Enterococcus spp.*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Escherichia coli (E. coli)*, *Staphylococcus aureus*, *Enterobacter spp.*, and *Klebsiella spp.*

Predictors of positive bile culture. The results of cross tabulation and calculation of chi-square tests have

shown a statistically significant association between a positive bile culture and patient age (*p*=0.000), gender (*p*=0.011), presence of associated morbidities (*p*=0.006), complicated gallstone disease (*p*=0.000), and the performance of preoperative ERCP (*p*=0.000). There was, however, no statistically significant association between any of these variables and the type of organism isolated. More importantly, there was no statistically significant association between bile culture results and postoperative septic complications. Table 4 shows the results of multinomial logistic regression analysis between the results of bile culture (dependent variable) and age, gender, preoperative ERCP, complications of LC, presence of associated disease, and indications of LC (independent variables). Age and preoperative ERCP were the only significant predictors for bile infection.

Table 1 - Indications for surgery and clinical findings among operated patients.

Indications/findings	n (%)
<i>Indications for surgery</i>	
Cholelithiasis	993 (79.6)
Acute cholecystitis	221 (17.7)
Jaundice	34 (2.7)
Total	1248 (100)
<i>Clinical findings</i>	
Associated morbidities	513 (41.1)
Preoperative ERCP	132 (10.6)
Postoperative septic complications	25 (2)

ERCP - endoscopic retrograde cholangiopancreatography

Table 2 - Results of bile culture.

Result	n (%)
Negative culture	998 (80)
<i>Positive culture</i>	
Gram negative bacteria	134 (53.6)
Gram positive bacteria	73 (29.2)
Mixed culture	43 (17.2)
Total	250 (100)

Table 3 - Types of organisms isolated from bile cultures.

Isolated organisms	n (%)
<i>Gram negative bacteria</i>	
<i>Pseudomonas aeruginosa</i>	45 (18)
<i>Escherichia coli</i>	37 (14.8)
<i>Enterobacter species</i>	23 (9.2)
<i>Klebsiella species</i>	15 (6)
Others*	14 (5.6)
Total	134 (53.6)
<i>Gram positive bacteria</i>	
<i>Enterococcus species</i>	46 (18.4)
<i>Staphylococcus aureus</i>	27 (10.8)
Total	73 (29.2)
Mixed cultures [†]	43 (17.2)
Total	250 (100)

*Includes *Acinetobacter species*, *Morganella species*, *Citrobacter species*, *Serratia species*, and *Salmonella species*.
[†]two or more genera of bacteria all being Gram positive or Gram negative in nature, or a combination of Gram positive and Gram negative bacteria

Table 4 - Multinomial logistic regression analysis between bile culture results (dependent variable), and several independent variables.

Variable	χ^2	df	P-value
Age	53.048	4	0.000
Gender	2.159	4	0.706
Preoperative ERCP	84.050	4	0.000
Complications	2.494	4	0.646
Associated morbidities	5.746	4	0.219
Indications for surgery	20.088	12	0.065

ERCP - endoscopic retrograde cholangiopancreatography,
 χ^2 - chi square test, df - degrees of freedom

Discussion. The rate of positive bile culture varies significantly between different studies, and it was reported to range from <10->70%.^{9,15} This variation is believed to be due to the selection of patients being influenced by the presence of complications particularly cholecystitis, common bile duct disease, and cholangitis,⁷ as well as the site of sampling from the gallbladder.²² Uncomplicated gallbladder stones on the other hand, are associated with approximately 20% positivity of bile culture,^{17,19} which is quite similar to the rate of positive bile culture in this study.

Whereas, *Enterococcus spp.* was the most frequently isolated organism in a finding that was also reported by Brody et al,¹¹ Gram negative bacteria were the predominant cause of bile infection in this study, which is in agreement with most published studies.^{6,9,12} A significant difference exists however, in the types of Gram negative bacteria isolated. Whereas, most studies reported *E. coli*, *Enterobacter spp.*, and *Klebsiella spp.* as the organisms most commonly encountered,^{6,9,12} *P. aeruginosa* was the most frequently isolated Gram-negative organism in this study. Similar findings were reported by Chunhamaneewat and Punyagupta,¹² and Al-Abassi et al.¹⁵ Since *P. aeruginosa* is not a frequent member of the resident flora in humans, it is legitimate to assume that infection was acquired intra-, or postoperatively. Mixed bile cultures were detected in 43 (17.2%) patients in this study, most of whom were elderly, suffering from co-morbidities, or have complicated gallstone disease. Mixed cultures may comprise an important fraction of positive bile cultures, and they represented approximately 40% of the positive bile cultures reported by den Hoed et al.¹⁹

This study attempted to determine the predictors of positive bile cultures in patients with gallstones, and it was demonstrated that many factors are significantly associated with positive bile culture. These factors includes gender - where women are more likely to develop bile infection, age - where the elderly are at increased risk, presence of associated morbidities, complicated gallstones, and the performance of preoperative ERCP. These finding are in agreement with the results of numerous studies.^{4,7,8,10-12,14,16} However, only age and preoperative ERCP were found by multinomial logistic regression analysis to be significant predictors of bile infection. Preoperative ERCP effect is probably due to sphincterotomy and ascending contamination of bile duct. Moreover, some of these patients have common bile duct stones that have been documented, similar to associated morbidities, to represent a risk factor for bile infection.^{7,8,15}

Controversy still surrounds the relationship between positive bile cultures and wound infection. Whereas some authors could not demonstrate a positive

correlation,^{6,15,18,19} others have reported an increased incidence of wound infection in cases of positive bile culture.^{8,16} No statistically significant correlation could be established in this study. Postoperative septic complications were observed only in 25 (2%) of the patients included in this study, which is a low rate compared to rates reported by some authors,^{12,14} but lower than that reported by many others.^{6,8,12-14,23} It has been documented however, that LC is associated with a lower incidence of septic complications compared to open cholecystectomy.^{12,14} Regardless of positive bile cultures, LC septic complications were reported to be associated with the same risk factors that increase the rate of bile infection as age, open surgery, urgent surgery, endoscopic interventions, hypertension, acute cholecystitis and others.^{16,19} The small number of postoperative complications that develop in this study did not allow for the assessment of risk factors. The prophylactic administration of antimicrobial agents might have contributed to such low rate of infectious complications.

This study being retrospective in nature, however, does not permit proper assessment of the preoperative prophylactic use of antimicrobials. This issue remains controversial as some authors recommend antibiotic prophylaxis, especially for high risk patients.^{10,13,14,17} Nonetheless, it was reported that antibiotic prophylaxis does not affect the incidence, or severity of postoperative infections.^{23,24} However, Tocchi et al,²³ Chang et al,²⁴ and den Hoed et al¹⁹ questioned the efficacy of antibiotic prophylaxis, whereas Wells et al,¹⁷ Grande et al,¹⁸ Sattar et al,⁶ and Galili et al,¹⁶ advocated preoperative prophylaxis. Moreover, Al-Abassi et al¹⁵ ascribed the very low rate of infective complications to their aggressive antibiotic regimen in the management of high risk patients. An acceptable approach would probably be to limit prophylactic antibiotics to patients with risk factors for bile infection.^{8,10,14}

In conclusion, the bile of patients with gallstone disease may harbor microorganisms, particularly in elderly patients, those who undergo preoperative ERCP, and those with complicated disease or associated morbidities, but these do not predispose for postoperative septic complications that may be prevented in high risk patients by preoperative antibiotic prophylaxis. However, a well-controlled prospective study to assess the value of antibiotic prophylaxis and its effect on post-operative complications is recommended.

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