

## Original Article

## BACTERIOLOGICAL STUDY OF BILE IN PATIENTS WITH CHOLELITHIASIS

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**Objective:** To determine the aerobic microbial flora in bile of patients having cholelithiasis.

**Methods:** Study included seventy patients of cholelithiasis, out of which 56 (80%) underwent open cholecystectomy, while 14 (20%) were operated by laparoscopic cholecystectomy. Bile for culture was taken per operatively in all patients.

**Results:** Mean age of patients was  $44.17 \pm 13.20$  and majority of patients were in age group 35-72. Female to male ratio was 3.4:1. Pain in right hypochondrium was major presenting feature in 65 patients (93%). Mean pain duration was  $26.21 \pm 23.44$  months. Bile culture was positive in 25 (36%) patients. 13 (62%) culture positive patients were diabetics. Postoperatively 7 patients (10%) developed fever while 4 patients (5.7%) developed wound infection.

**Conclusion:** The frequency of positive bile culture in patients of cholelithiasis was 36%, in this series, commonest organisms being *E. coli* and *Klebsiella*. Wound infection 5.7% could have been due to endogenous or exogenous contamination. As all patients who developed wound infection were culture positive in this series, peri-operative antibiotic prophylaxis is recommended in patients undergoing biliary surgery.

**Keywords:** Gallstones, Open and Laparoscopic Cholecystectomy, Bile Culture.

### Introduction

Gallstones are present in about 10-15% of the adult population. Between 1-4% become symptomatic each year.<sup>1</sup> Among the digestive diseases requiring hospitalization, gallstones are the most common.

Bile formation is one of the most sophisticated functions of liver and it is also one of the most readily disturbed. It constitutes the primary pathway for the elimination of bilirubin, excess cholesterol and xenobiotics.<sup>2</sup> when bile leaves the liver; it is composed of water, bile salts, bile pigments, cholesterol and fatty acids.<sup>3</sup>

Gallstones are the most common biliary pathology responsible for more than 95% of biliary tract diseases<sup>4</sup> and is the commonest indication for abdominal surgery.<sup>5</sup> Different factors have been implicated in the causation of gallstones amongst which infection of the bile is also important factor.<sup>6</sup> Inflamed gallbladder has markedly altered permeability, which permits absorption of bile acids and movement of inorganic salts into the gallbladder lumen. The role of excessive cellular debris and increased protein secretion, which occurs in response to inflammation, may be present. Most gallstones are composite in nature. Bacteria can be found in most pure stone (i.e. those whose structure consists more than 90% cholesterol).<sup>7</sup>

The natural history of gallstones is unknown. It is likely that brown pigment stones can evolve in their

chemical composition after termination of the infection process that initiate their formation, and may further develop into either mixed or nearly pure cholesterol stones.<sup>7</sup> Gallstones may lead to acute calculus cholecystitis or chronic cholecystitis.<sup>3</sup> Acute cholecystitis is a chemical or bacterial inflammation of the gallbladder. In approximately 95% of patients with acute cholecystitis, stones are present in the gallbladder. Acute cholecystitis is caused by gallbladder outlet obstruction, almost always by a stone.<sup>8</sup> Chronic cholecystitis may follow an acute episode or it may occur primarily without antecedent acute cholecystitis. It is almost always associated with gallstones.<sup>8</sup> Treatment modalities for cholecystitis and gallstones are; laparoscopic cholecystectomy, minicholecystectomy, or conventional open cholecystectomy.<sup>9</sup> Amongst treatment options, laparoscopic cholecystectomy has rapidly become the "gold standard" for uncomplicated symptomatic gallstone disease.<sup>10</sup> Cholecystectomy is a clean contaminated operation according to the surgical wound classification.<sup>3</sup> In about 30% of the patients with cholelithiasis, bacteria can be cultured either from the bile or from the wall of the gallbladder. The biliary infection can be caused by any type of bacteria ranging from aerobic gram positive or gram negative to anaerobic organisms. Aerobic organisms cause 94% of biliary tract infection while anaerobic organisms cause the rest. Bacteria are commonly found in inflamed gallbladder and in patients with cholelithiasis, whereas evidence suggests

Than normal bile is sterile.<sup>6</sup> It is difficult to ascertain that whether bacterial infection of bile arose from stone formation or vice versa. Although the exact contribution of bacteria in lithogenesis is not known, it is important for the clinician to realize that most gallstones are likely to be colonized by bacterial biofilm, even though the bile may be culture negative.<sup>7</sup> so in the treatment of symptomatic gallstone disease (i.e. cholecystectomy) perioperative antibiotics are being given to avoid infective postoperative complications.<sup>9</sup> The aim of study is to determine the presence of aerobic bacteria in patients suffering from chronic cholecystitis as, to effectively treat the biliary tract infections and postoperative wound infections, a comprehensive knowledge of bacteriology of bile is essential.

## Methods

This study was done in Department of Surgery Unit I, SIMS Services Hospital Lahore. Seventy admitted patients were selected from Surgical Unit I of Services Hospital Lahore. Diagnosis was made on the basis of history, clinical examination and investigations. Detailed history including recurrent attacks of pain right hypochondrium or pain epigastrium, episodes of biliary colic, flatulence, dyspepsia, fever, nausea, vomiting, jaundice were recorded. Examination findings including, tachycardia, temperature, jaundice, tenderness in right hypochondrium, Murphy's sign were also recorded. Complete blood count, liver function tests, random blood sugar level, abdominal ultrasonography was carried out. Patients were asked to sign an informed consent. All Patients underwent cholecystectomy, during which bile sample was collected under aseptic measures and sent to Microbiology Department of SIMS Services Hospital, Lahore for aerobic bacterial culture. Wound examination was done on 2<sup>nd</sup> and 5<sup>th</sup> day for any type of infection. All that information including bile culture report (positive or negative) was recorded on a Performa.

The collected data was entered and analyzed by using computer program SPSS version 11. The variables to be analyzed were age, gender, symptoms (recurrent attacks of pain right hypochondrium or pain epigastrium, episodes of biliary colic, flatulence, dyspepsia, fever), examination findings (tachycardia, temperature, jaundice, tenderness in right hypochondrium, Murphy's sign) and investigations (complete blood count, liver function tests, random blood sugar

level, abdominal ultrasonography and aerobic culture of bile).

Mean and standard deviation was calculated for age and duration of symptoms. Gender, type of symptom, bile infection (whether culture positive or negative), type of aerobic flora (in case of positive culture) were expressed as frequency and percentage. Bile infection and type of aerobic flora (*Escherichia coli*, *Klebsiella*, *Streptococci*, *Staphylococci*, *Pseudomonas*, and *Proteus*) were presented as frequency distribution table.

## Results

This study included seventy patients of cholelithiasis. Out of these 56 (80%) patients underwent open cholecystectomy while 14 (20%) patients had laparoscopic cholecystectomy. This study was conducted at Services Hospital Lahore, Surgical Unit-I.

The age of the patients ranged from 12 to 72 years. The mean age of the patients was  $44.17 \pm 13.20$  years. Of these 9 (13%) patients were in the age group between 12-32 years, 35 (50%) patients were in age group between 33-52 years, while 18 (26%) patients were in age group between 53-72 years and 8 (11%) patients were in age group 72 years or above.

There were 16 (23%) males and 54 (77%) female patients. Female to male ratio was 3.4:1. Abdominal pain was the major presenting feature which was located in right hypochondrium and/or epigastrium in 65 (93%) patients, 5 (7%) patients had no history of abdominal pain. Pain duration ranged from 1-120 months. The mean of pain duration was  $26.21 \pm 23.44$  months. Of these 50 (71%) patients had pain duration between 1-30 months, 13 (19%) patients had pain duration between 31-60 months, while 4 (6%) patient had pain duration between 61-90 months and 3 (4%) patients had pain duration between 91-120 months.

Dyspepsia was the next common presenting symptom reported by 47 (67%) patients, while 23 (33%) patients had no dyspepsia. The difference was statistically significant ( $p < 0.05$ ). The nausea was present in 42 (60%) while this complaint was absent in 28 (40%) patients. The difference was not statistically significant ( $p > 0.05$ ). Regarding clinical signs, temperature of patients ranged from 98-101F°. The mean temperature of patients was  $98.80 \pm 0.70$ . Of these 59 (84%) patients were in the temperature range between 98-99F°, while 11 (16%) patients were in the temperature range between 100-101F°. Deep tenderness was present in 55 (79%) patients, while 15 (21%) patients had no deep tenderness. The gall bladder was palpable in 2 (3%) patients while 68

(98%) patients had no palpable gallbladder. There were 14 (20%) patients who had single calculus in their gallbladder, while 56 (80%) patients had multiple calculi, as evident by abdominal ultrasonography. Open cholecystectomy was performed in 56 (80%) patients while laparoscopic cholecystectomy was done in 14 (20%) patients. Per operatively increase gallbladder wall thickness was found in 12 (17%) patients, while it was normal in 58 (83%) patients. Bile culture was positive in 25 (36%) of patients, of these 5 (7%) patients were male while 20 (28%) patients were female. 45 (64%) patients had negative bile culture, of these 11 (16%) were male patients, while 34 (49%) were female patients.

**Table-1:** Age distribution of patients (n=70).

Age in years	Frequency	Percentage
12 -32	09	13.0
33 - 52	18	26.0
53 - 72	35	50.0
> 72	08	11.0
Total	70	100.0

**Table-2:** Distribution of Culture Sensitivity with Sex of Patients (n=70).

Culture Sensitivity	Frequency Percentage	Male Percentage	Female Percentage
Positive	25 (36%)	05 (7%)	20 (28%)
Negative	45 (64%)	11 (16%)	34 (49%)

**Table-3:** Frequency of Organism in Positive Culture Bile of Patients (n=25)

Culture Type	Frequency	Percentage
E.Coli	10	40.0
Klebsiela	06	24.0
Pseudomonsa	05	20.0
Proteus	02	08.0
Staphaureus	02	08.0

**Table-4:** Distribution of culture positivity and complications with Sex (n=25).

Culture positive and complications	Sex	
	Male Percentage	Female Percentage
Fever	2 (8%)	5 (20%)
Wound Infection	1 (4%)	3 (12%)
No complications	2 (8%)	12 (48%)

Frequency of organisms in positive bile culture patients showed 10 (40%) E. coli, 6 (24%) Klebsiella, 5 (20%) Pseudomonas, 2 (8%) Proteus and 2 (8%) Staph aureus. 7 (10%) patients suffered from postoperative fever, while 4 (6%) patients got wound infection and 59 (84%) patients had no postoperative complications.

All patients who suffered from fever had positive bile culture, among them 2 (8%) were male patients while 5 (20%) were female patients, 1 (4%) male patient got wound infection and 3 (12%) female patients got wound infection. There were 13 (52%) culture positive patients who were diabetics among them 2 (8%) were male patients and 11 (44%) were female patients, while 12 (48%) patients with positive bile cultures were non-diabetics.

## Discussion

Cholecystitis and cholelithiasis are prevalent in certain regions of the world and are quite rare at other places.<sup>13</sup> Hence these are sometimes called South Western American diseases and has been reported in 54% of the adults above 21 years of age.<sup>7</sup> Normal biliary tract and bile is sterile in healthy population, however, bacteria are frequently present in biliary tract disease and may lead to septic complications.<sup>8</sup> A bacterial cause of cholecystitis has been proposed and positive bile cultures have been noted in 46% of patients with acute cholecystitis.<sup>7</sup> In one study from Germany, using molecular genetics methods, bacteria could be found in most pure cholesterol stones (i.e. those whose structure consists of more than 90% cholesterol).<sup>14</sup> It is suggested that bile infection by E. Coli, in addition to bile stasis, plays a crucial role in the pathogenesis of brown pigment stones.<sup>15</sup> Interest has continued to abound in the role of infection in cholelithiasis. Two fallacies, however, exist in this regard. Firstly, the culture of the organism from the bile at the time of the operation does not necessarily indicate a cause effect relationship between the infective micro-organism and lithogenesis, as infection may be secondary to calculous formation. Secondly, the failure to isolate organism from bile also does not indicate that the etiology is unrelated to the infection as it is well known that organisms which have initiated the stone precipitation may not persist in the viable form in the bile till surgery. This study shows that this disease is much more common in females as compared to the males. The mean age incidence in this series is 44 years. Iqbal et al<sup>16</sup> reported maximum number of patients with cholelithiasis between the age of 20-30 years with the highest

incidence in 61-75 years of age. In this series, the positive bile culture was 36%, which is considerably higher than that reported by Yaqin and Sultan.<sup>17</sup> However, more recently Al-Abbasi et al<sup>18</sup> have reported an incidence of 9%. Harbi<sup>28</sup> reported 25% and Pokharel et al<sup>19</sup> reported 8%. Hazrah et al<sup>20</sup> reported from India that the incidence of bacteria to be very high, ranging from 20 to 80% depending on the kind of gallstone present. The frequency of positive bile culture also differ in acute and chronic calculous cholecystitis. Linhares et al<sup>21</sup> showed positive bile culture in 68% of patients. Karamarkovic et al have showed 79% positive culture in acute and 18% positive culture in chronic cholecystitis.<sup>22</sup> Chang et al<sup>23</sup> have showed 47% positive bile culture in acute and 17% in chronic cholecystitis. Therefore infection is likely to range from 8 to 80% which corroborates with the present finding. E. coli was found to be the commonest organism in this study as has already been reported by previous studies, however, klebsiella was reported by Sabir.<sup>24</sup> In one of the Saudi studies, the most common organism isolated were E. coli (28%). Pseudomonas (9.4%) klebsiella (6.3%) Staph aureus (12.4%).<sup>12</sup> Petakovic et al<sup>25</sup> showed E. Coli (55%) klebsiella and Staphylococcus (10% and 34%). The importance of the predominance of E. coli is seen by the fact that older studies have shown glucuronidase enzymatic activity of E. coli to have a role to play in calcium bilirubinate gallstone formation. The other organisms found in our study were klebsiella, Pseudomonas, Proteus and Staph aureus. These are quite consistent with other series as Ohdan et al showed E. coli, Klebsiella and pseudomonas.<sup>11</sup> The frequency of postoperative wound infection in this study of 5.7% is consistent with study by Sattar et

al<sup>7</sup> in 2007 which showed postoperative wound infection of 4.9%. But in his study half of the patients who developed postoperative wound infection were culture positive while half were not. In present study all patients who developed wound infections were culture positive. The low frequency of wound infection in this series may be due to the fact that all the patients were operated electively and had perioperative prophylactic course of antibiotics. According to Rehman and Anson<sup>26</sup> perioperative antibacterial prophylaxis reduces surgical site infections significantly (6% in antibacterial prophylaxis group versus 15% in control group in which no antibiotics were given). The limitations of this study were that antibiotic was injected intravenously, about 10-15 minutes before the bile sample was collected, and therefore it may be argued that the bile sample could have some quantity of the antibiotic. The pus from the infected wound was minimal; therefore role of biliary bacteria in wound infection cannot be concluded. Anaerobic cultures were not included in this study because anaerobes are present in only 6% of patients with cholelithiasis.

### Conclusion

The frequency of positive bile culture in patients of cholelithiasis was 36%, in this series, commonest organisms being E. coli and Klebsiella. Wound infection 5.7% could have been due to endogenous or exogenous contamination. As all patients who developed wound infection were culture positive in this series, peri-operative antibiotic prophylaxis is recommended in patients undergoing biliary surgery.

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### References

- Gurusamy KS, Smaraj K. Early versus delayed laparoscopic cholecystectomy for acute cholecystitis. *Cochrane Database Syst Rev* 2006; 4: CD00440.
- Crawford JM. The liver and the biliary tract. In: Kumar V, Cotron RS, Robbins SL editors. *Pathologic basis of disease*. 7th ed. Philadelphia: WB Saunders; 2004. 516-56.
- Russel RCG. The gallbladder and bile ducts. In: Russel RCG, William NS, Bulstrode CJK, editors. *Bailey and Love's Short practice of surgery*. 24th ed. London: Arnold; 2004. 1094-11-13.
- Crawford JM. The liver and biliary tract. In: Robbins pathological basis of disease. 5th ed. Philadelphia: WB Saunders 1994; 831-96.
- Kirk RM. Biliary tract. In: Kirk RM editor. *General surgical operations*. 3rd ed. London: Churchill Livingstone, 1994: 319.
- Cuschieri A, Bouchier IAD. The biliary tract. In: Cuschieri A, Giles GR, Moosa AR. Editors. *Essential surgical practice*. 2nd ed. Edinburgh: Butterworth-Heinemann, 1988; 1020-75.
- Sattar I, Aziz A, Rasul S, Mehmood Z, Khan A. Frequency of infection in cholelithiasis. *J Coll Phys Surg Pakistan* 2007; 17: 48-50.
- Majid A, Haider W, Syed MA, Waheed A. Bacteriological examination of bile in chronic cholecystitis. *Pak Postgrad Med J* 2002; 13: 91-4.
- Cushieri A. Disorders of the liver. In: Cushieri A, Steel RJC, Moosa

- AR. Editors. Essential surgical practice. 4th ed. London: Arnold; 2002; 375-452.
10. Cheema AM, Munir A, Zahid M. An experience of laparoscopic cholecystectomy at Lahore General Hospital. *Biomedica* 2001; 17: 32-6.
  11. Ohdan H, Oshiro H, Yamamoto Y. Bacteriological investigation of bile in patients with cholelithiasis. *Surg Today* 1993; 23: 390-5.
  12. Al-Harbi M, Osoba AO, Mowallad A, Al-Ahmad K. Tract microflora in Saudi patients with cholelithiasis. *Trop Med and International Health* 2001; 6: 570-4.
  13. Ballal M, Jyothi KN, Antony B, Arun C, Prabhu T, Shivananda PG. Bacteriological spectrum of cholecystitis and its antibiogram. *Indian J Med Microbiol* 2001; 19: 212-4.
  14. Swidsinski A, Lee SP. The role of bacteria in gallstone pathogenesis. *Front Biosci* 2001; 6: 93-103.
  15. Lee DK, Tarr PI, Lee SP. Bacterial DNA in mixed cholesterol gallstones. *Am J Gastroenterol* 1999; 94: 3502-6.
  16. Iqbal P, Sial K, Sial E. Gallstone disease: an experience at Civil Hospital, Karachi. *Med Channel* 2001; 7: 17-20.
  17. Yaqin H, Sultan G. Results of culture of gallbladder, bile and gallstones. *J Pak Med Assoc* 1978; 28: 31-2.
  18. Al-Abassi AA, Farghaly MM, Ahmed HL, Mobasher LL, Al-Manee MS. Infection after laparoscopic cholecystectomy: effect of infected bile and infected gallbladder wall. *Eur J Surg* 2001; 167: 268-73.
  19. Pokharel N, Rodrigues G, Shenoy G. Evaluation of septic complications in patients undergoing biliary surgery for gallstones in a tertiary care teaching hospital of South India. *Kathmandu Univ Med J* 2007; 5: 371-3.
  20. Hazrah P, Oahn KTH, Tewari M, Pandey AK, Kumar K, Mohapatra TM et al. The frequency of live bacteria in gallstones. *HPB* 2004; 6: 28-32.
  21. Linhares MM, Paiva V, Castelo Filho A, Granero LC, Pereira CA et al. Study of preoperative risk factors for bacteriobilia in patients with acute calculosis cholecystitis. *Rev Assoc Med Bras* 2001; 47: 70-7.
  22. Karamarkovic AR, Popovic N, Bajec D, Jeremic V, Dukic V, Radenkovic D et al. Relation of pathoanatomic substrate and incidence of bacterial infection in the acute biliary tract disease. *Srp Arh Celok Lek* 2005; 133: 52-7.
  23. Chang WT, Lee KT, Wang SR, Chuang SC, Kuo KK, Chen JS et al. Bacteriology and antimicrobial susceptibility in biliary tract disease: an audit of 10-year's experience. *Kaohsiung J Med Sci* 2002; 18: 221-8.
  24. Sabir O. Infected bile in gallbladder in cholelithiasis (dissertation). Karachi: Coll Physicians Surg Pak 1996; 131: 389-94.
  25. Petakovic G, Korica M, Gavrilovic S. Bacteriologic examination of gallbladder contents. *Med Pregl* 2002; 55: 225-8.
  26. Rahman MH, Anson J. Perioperative antibacterial prophylaxis. *Pharmaceutical J* 2004; 272: 743-5

## Medical News

### VACCINE CANDIDATE OFFERS PROTECTION FROM ZIKA VIRUS IN ANIMAL TESTS

A newly developed vaccine against Zika virus induces a rapid and long-lived immune response in mice and non-human primates, a Nature paper reports. Although further tests are needed to assess the vaccine's effects on pregnant animals and unborn fetuses, it remains a promising candidate for further development in the global fight against Zika virus.

Drew Weissman and colleagues devised a novel vaccine based on a modified messenger RNA that encodes two different Zika virus proteins. Mice and rhesus macaques given a single, low dose of the vaccine developed neutralizing antibodies against Zika virus within 2 weeks. Critically, the immunity persisted when vaccinated animals were challenged with Zika virus. Mice and macaques remained protected at 5 months and 5 weeks post-vaccination, respectively.

The new vaccine has several potential advantages over previously described alternatives. Delivered by lipid nanoparticles by injection just below the skin, it is easy to administer. Moreover, while some other vaccines require two immunizations with large doses, a single low dose of the new vaccine offers lasting immunity in animal models, potentially making it more cost-effective and scalable (just a single contact with healthcare workers would be needed). Made from mRNA, the vaccine is also non-replicating and cannot integrate into the host genome, easing safety concerns.

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