

**A clinical study of gall bladder disease and its relationship to bacteriology of biliary tract**Dr Siddharth Singh¹ (MS, FMAS, FIAGES, DMAS), Dr Amitabh Satsangi (MBBS)²¹Associate professor, department of general surgery, G.S.V.M Medical College and Associated LLRH hospital.²Post graduate 3rd year department of general surgery, G.S.V.M Medical College and Associated LLRH hospital**Correspondence Author:** Dr Amitabh Satsangi, Department of general surgery, G.S.V.M Medical College and Associated LLRH hospital, India.**Type of Publication:** Original Research Paper**Conflicts of Interest:** Nil**Introduction**

Gall stone disease is one of the common surgical conditions requiring surgery in elective setup⁸. Most are asymptomatic but still gallstone disease contribute substantially to health care costs and its complications are sometimes life threatening.

The pathogenesis of gall stone is complex and it appears to be influenced by genes and environment and their interactions.¹¹⁻¹⁵

Among the important environmental factors in the pathogenesis of gall stones is the involvement of bile bacteria.⁷

Under conditions of normal bile flow, bacteria in the biliary system are of no clinical significance. Upon bile duct obstruction, bacteria proliferate within the stagnant bile while biliary pressure increases. Eventually, the bacteria presumably translocate into the circulation causing a systemic infection. Acute cholangitis spans a continuous clinical spectrum and can progress from a local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndrome. Therefore, it is important to know the microbiological flora of the gallbladder before prophylactic antibiotics are given.¹⁵⁻¹⁸

Prophylaxis would be appropriate according to bacteria isolated from the bile and could prevent postoperative infections.

In the era of antibiotic resistance and the need for meticulous administration of antibiotics it is a dire need to justify antibiotic prophylaxis against biliary organisms, which makes it a necessity to show that the bile is colonized with bacteria.

The study of bacterial flora in surgical biliary diseases assumes profound importance as a result of studies which indicate that after biliary surgery, the incidence of infectious complications appeared to be forty times greater in patients with infected bile than in those with sterile bile (Chetlin S.H., 1988).

Hence, our main aims of study of biliary bacterial flora

- 1) To help in administration of preoperative antibiotics in a more selective and rational basis in patients undergoing biliary tract surgery.
- 2.) To make use of post-operative antibiotics in the more discriminate manner.
- 3.) To establish evidence of benefits of appropriately timed antibiotics in high risk patients.
- 4.) To determine incidence of positive bacterial cultures in bile and colonization rates in different segments of biliary tract.

Material and Methods

Biliary bacterial flora in surgical biliary tract diseases was studied and investigated in following manner.

Patients with pre-operative diagnosis of biliary tract disease were divided into four major groups on the basis of clinical presentation and preliminary investigation:

- Patients having jaundice with stones.
- Patients having jaundice but no stones.
- Patients having no jaundice but with stones.
- Patients having symptoms of biliary tract disease but no jaundice or stones.

Specimen of bile from gall bladder and CBD was aseptically collected in sterile tubes and sent to microbiology laboratory for culture and sensitivity of bacterial pathogens.

-Detailed clinical history was recorded.

-Then they were thoroughly investigated by routine investigations like Hb% TLC, DLC, BT, CT, urine examination, blood sugar and serum creatinine.

Specific investigations were also carried out in almost all cases including Oral cholecystogram, Ultrasonography , Liver Function Tests.

Method Used For Isolation of Bacterial Flora From Bile

The specimens were collected in aseptic containers and inoculated by standard procedures on liquid and solid media and incubated at 37°C temperature for 48 hours.

Various media used were

- Digest both
- Macconkey medium
- Blood Agar Medium
- DCA and
- Medium for appropriate organisms.

Bacterial pathogens were identified on positive culture cases by colony characteristics, morphology and biochemical tests whenever found necessary.

For anaerobic bacteria, one portion of specimen was inoculated on Robertson's cooked medium and liquid Thioglycolate medium and organisms identified by

morphology. Pure cultures were obtained by pre-treating the specimen with appropriate bactericidal substances which destroys unwanted bacteria. Organisms thus isolated were tested for antibiotic sensitivity by standard dose diffusion method. Antibigram was noted after overnight incubation. Administration of antibiotics was guided by A.S.T.

Observation

Observations have been carried out on 70 patients with various diseases of biliary tract, admitted in L.L.R. and associated hospitals from March 2015 to November 2017. Age of the patients studied ranged from 16 years to 70 years. The distribution of cases in various age group was as shown in the following table.

AGE GROUP	NO. OF CASES	PERCENTAGE
0-10	NIL	-
11-20	2	3.00
21-30	6	10.00
31-40	12	19.60
41-50	19	28.30
51-60	20	23.60
61-70	11	15.70
71-80	NIL	-
TOTAL	70	100

In our study, maximum number of cases was in the age group of 41 to 50 years (28%) as depicted by the above figures. Age group closely followed by age group 51 to 60 years (23%). Table 1

Table II Showing Sex Distribution Of The 70 Studied Cases.

SEX	NO. OF CASES	PERCENTAGE
FEMALES	62	89
MALES	8	11
TOTAL	70	100

Majority of the patients studied were females. Males constituted only 11% of the total cases. Table II

Table-III Distribution of Cases with Different Preoperative Diagnosis of the Various Biliary Tract Diseases In Relation To Presence or Absence of Jaundice.

WITHOUT JAUNDICE AND CBD STONE	48	69
WITH JAUNDICE AND CBD STONE	6	9
WITH JAUNDICE BUT WITHOUT STONE	8	11
WITHOUT JAUNDICE BUT WITH STONE	4	5.5
WITHOUT JAUNDICE OR STONE (ACALCULOUS CHOLECYSTITIS)	4	5.5
TOTAL	70	100

Majority of cases (69%) belonged to the category of ‘without jaundice and without C.B.D. stone’.

Table IV: Pattern of various Gall Bladder diseases observed.

PATTERN	NO. OF CASES	PERCENTAGE
ACUTE CALCULUS CHOLECYSTITIS	16	22.80
CHRONIC CALCULUS CHOLECYSTITIS	50	71.50
ACALCULOUS CHOLECYSTITIS	4	5.70
TOTAL	70	100

Majority of cases (71.50%) belonged to the category of chronic calculus cholecystitis.

Maximum percentage (80 %) of bile positive cases form ‘Acalculous cholecystitis’ category.

TABLE VI: Number of Sterile and Positive Cultures from Gall Bladder.

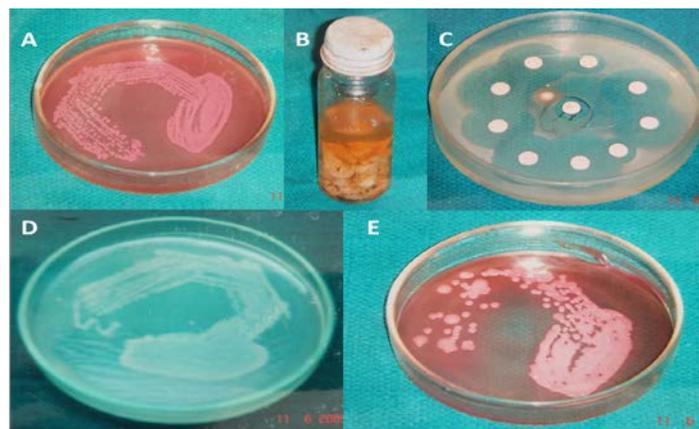
CULTURE REPORT	NO.	PERCENTAGE
STERILE	24	34.30
POSITIVE	46	65.70

Number of positive cultures were significantly higher than that of sterile cultures.

Table VII: Various Types Of Bacterial Flora Found In The Positive Biliary Cultures From Gall Bladder.

TYPES OF BACTERIAS	NO.	PERCENTAGE
E.COLI	24	52.20
KLEBSIELLA	8	17.40
PROTEUS SPECIES	3	6.50
STAPHYLOCOCCUS AUREUS	1	2.20
MIXED AEROBIC ANAEROBIC	9	19.50
TOTAL	46	100

E.coli was the commonest organism (52.2%) isolated from biliary culture of gall bladder followed by Klebsiella (17.40), proteus, staphylococcus aureus and other bacteria as shown in table VI.



A). E. COLI GROWTH B). ROBERTSON COOKED MEAT MEDIA C).CULTURE DISC SHOWING ANTIBIOTIC SENSITIVITY D).STAPHYLOCOCCUS AUREUS E). KLEBSIELLA GROWTH

TABLE VIII: Different Bacteria Are Isolated From Mixed Cultures.

Varietes	No. of cases	Percentage
E. COLI & KLEBSIELLA	7	77.80
E. COLI & STAPHYLOCOCCUS	1	11.10
E. COLI & PSEUDOMONAS	1	11.10
TOTAL	9	100

E.coli & Klebsiella, were found to be responsible for most of the mixed infections. Table VIII.

TABLE IX: Number Of Cases With Positive Aerobic, Anaerobic And Mixed (Aerobic – Anaerobic) Cultures

VARIETY OF CULTURE	NO. OF CASES	PERCENTAGE
Aerobic	45	97.20
Anaerobic	1	2.20
Mixed (aerobic and anaerobic)	Nil	-
Total	46	100

TABLE X: Incidence of Positive Bacterial Cultures as Regards to the Various Age Groups.

AGE - GROUP	NO. OF CASES	POSITIVE BACTERIA	PERCENTAGE
0-10	NIL	NIL	-
11-20	2	NIL	0
21-30	6	2	33
31-40	12	6	50
41-50	19	12	63
51-60	20	16	80
61-70	11	10	90
71-80	NIL	-	-
TOTAL	70		

The maximum number of positive bacterial cultures were from 61-70 years age group (90%). Table X

Table XI: Sex –Wise Positive Bacterial Cultures.

SEX	NO. OF CASES	POSITIVE	PERCENTAGE
MALE	8	6	75.00
FEMALE	62	40	64.50
TOTAL	70	46	

Bile of males was found to be more susceptible to bacterial infection as compared to females. Table XI

Table XII: Various Types of Surgical Procedures Performed In the Different Diseases of the Biliary Tract.

TYPE OF SURGERY	NO. OF CASES	PERCENTAGE
CHOLECYSTECTOMY ONLY	58	82.90%
CBD EXPLORATION ONLY	2	2.9%
CBD EXPLORATION WITH CHOLECYSTECTOMY	6	8.5%
BILIARY ENETERIC ANASTAMOSIS	4	5.70%
SPHINCTEROPLASTY	NIL	-
TOTAL	70	100%

Simple cholecystectomy was performed in the maximum number of cases 82.90 % . Table XII

TABLE XIII: Culture Results In Relation To History Of Preoperative Fever.

VARIETY	NO. OF CASES	PERCENTAGE
PATIENTS	70	100.00
POSITIVE CULTURE	46	65.00
NEGATIVE CULTURE	24	34.28
FEBRILE	12	17.15
POSITIVE CULTURE AND FEVER	10	21.6
NEGATIVE CULTURE AND FEVER	2	8.3

Number of cases with positive culture and history of preoperative fever were more than that of negative culture and history of fever. Table XIII.

TABLE XIV: Culture Report of Bile from Common Bile Duct.

CULTURE REPORT	NO.	PERCENTAGE
POSITIVE	5	62.5
NEGATIVE	3	37.5
TOTAL	8	100

Number of C.B.D culture cases which were positive was more than the negative culture. Table XIV

TABLE XV: Number of Positive C.B.D Bile and Gall Bladder Bile Cultures.

POSITIVE CULTURES	NO.	PERCENTAGE
POSITIVE	51	65
NEGATIVE	27	35
TOTAL	78	100

Number of cases with positive C.B.D bile and gall bladder bile cases were more than negative cultures.

Table XV

TABLE XVI: Number of Cases with Positive CBD Bile and Negative Gall Bladder Bile.

CASES	NO.	PERCENTAGE
TOTAL NO. OF POSITIVE C.B.D BILE CASES	5 OUT OF 8	62.5
POSITIVE C.B.D BILE AND NEGATIVE GALL BLADDER BILE	1	

Number of cases with positive CBD bile and negative gall bladder bile was 1. Table XVI

TABLE XVII: Number of Cases With Positive Gall Bladder Bile And Negative C.B.D. Bile.

CASES	NO.	PERCENTAGE
TOTAL NO. OF POSITIVE GALL BLADDER CASES	46	65.11
POSITIVE GALL BLADDER BILE AND NEGATIVE CBD BILE.	2	2.86

TABLE XVIII: Various Types of Bacterial Flora Found In the Positive Common Bile Duct Cultures

TYPE OF BACTERIA	NO.	PERCENTAGE
E.COLI	2	40
KLEBSIELLA	-	-
PROTEUS	1	20
MIXED AEROBIC	2	40
TOTAL	5	100

E.coli and mixed aerobic bacteria’s were found to be prevalent in equal number of cases i.e. 40% each.

Table XVIII.

TABLE XIX: Relationship of Biliary Bacterial Flora with Post Operative Wound Infection.

NO. OF GALL BLADDER = BILE POSITIVE CASE	-	46
WOUND INFECTION OCCURRED IN 22	-	48 % CASES
NO. OF GALL BLADDER BILE NEGATIVE CASES	-	24
WOUND INFECTION OCCURRED IN 6 CASES	-	25%

Discussion

This study was undertaken to find out the incidence of positive bile culture and presence or absence of different types of bacterial flora in various types of surgical biliary tract diseases.

Our study may well be appointed to the various sinister complications of biliary tract surgery, thereby altering the surgeon to take proper steps before and after embarking upon surgery.

With advancement in technique and attainment of expertise, the procedure is gaining immense popularity amongst surgeons. The main criteria which can be attributed to this popularity are easy technique, quick results and no discomfort to the patient.

This study, was not always infallible, the credence to this belief coming from some false positive and false negative case reports. Nonetheless, the immense importance of this simple reports study cannot be overlooked.

Our study included 70 cases with various biliary tract disease , who were chosen randomly from the patients admitted in our wards. Patients were first clinically examined, and then subjected to various investigations. After this they were categorized as:

- Patients with jaundice and common bile duct stones.
- Patients without jaundice and common bile duct stones.
- Patients with jaundice but without stone.
- Patients without jaundice or stone (Acalculous cholecystitis).

The maximum number of cases belonged to the group without jaundice or common bile duct stones i.e. 48 cases (68.578%).

Maximum number of cases lay in age group of 51-60 Years, with a steady decline upto age of 31 yrs and drastic decline in lesser age group. Also, there was a decline in number of cases beyond this age group. This finding, though not identical, is commensurate with the findings of the study conducted by J. Suresh Babu et al (1988).

Average age of the patients was found Average to be 55.5 Yrs. Which is different from the study conducted by Shrestha KR et al (2014) where it was 28.09 years and Pratik M. Parekh et al (2015) where it was between 41-50 yrs . However, this finding Is very near to that of RG Willis et al (1978) where the average age group was found to be 51.6 yrs.

Our finding also commensurates with the various other studies conducted by different workers.

As regards the sex distribution, the percentage of female patients in our study was 88.6 as compared to males which was 11.4,i.e. F:M ratio of 7.7:1, this was significantly different from the study by Yun Lu et al

(2002) (F : M= 2.5: 1). In Alaattin Öztürk et al. (2012) study, 15 a total of 114 patients were included in this study. Ages ranged from 17 to 86 years with a mean age of 48.6 ± 16.8 . 81 patients (71.1%) were women and 33 (28.9%) were men. (Female:Male=2.5:1).

This was also different from the various other studies conducted. This could be explained from the fact that Indian men, being bread winners of the family avoid admission for surgery as long as possible.

The pattern of the various Gall Bladder diseases studied showed the maximum incidence of chronic calculous cholecystitis (71.50%), followed by acute calculous cholecystitis (22.80%) and Acalculous cholecystitis (5.70%) No incidence of acute emphysematous cholecystitis was observed. In the literature, most of the studies are silent on this part.

Cultures of bile from Gall Bladder were sent in all the selected 70 patients and found to be positive in 46 patients (65.70%) as against 24 negative culture as (34.30%). This was again commensurate with that of Bose et al who had studied 55 patients, of which 60% had final: positive bile at time of surgery and also with that of Yun Lu et al (2002) who studied 59 patients of which 72.9% had positive bile at the time of surgery. Other studies also corroborate this finding, though some highly contradictory studies are also seen.

Different patterns of colonization by biliary bacteria were noted. E. Coli was found to be Positive in more than 50% of the positive cultures in our study. This study is strongly favoured by studies conducted by Tejero A et al (1990), Bapat RD et al (1996), Acharya Suri et al.(2009), Pratik M. Parekh et al (2015), The other organisms which were detected were klebsiella (17%), proteus (7%), staphylococcus and Anaerobic (2%) each.

Almost 20% of cases consisted of mixed aerobic infections. No Pseudomonas, Haemolytic streptococci or

Acinetobacter were found. Mixed aerobic growths were found in 17% by Fukunaga et al (1973), 15 % case conducted by Babu et al (1988), 19 % cases conducted by Bapat Rd et al (1996), 27 % cases conducted by Nielsen ML et al (1996), 7% by Chang WT et al (2002) and 45.8% cases by Yun Lu et al (2002). Various studies have been conducted all showing vast difference regarding presence of the various bacterias apart from E.coli .

Babu et al(1988) found E. coli in 40% cases, staphylococcus epidermitis in 10% cases, klebsiella in 8%, pseudomonas in 6%, haemolytic streptococci in 5% and anaerobic in 10 % cases.

Culture reporting by Chang WT et al (2002) revealed that the commonest organism cultured were gram negative bacteria (74%) in which Escherichia coli (36%) and Klebsiella (15%) were most commonly found, followed by Gram positive (15%) bacteria such as Enterococcus (6%), Staphylococcus (3%), Streptococcus (2%). Bacteroides (5%) and Clostridium (3%) were occasionally found anaerobes (9%). Polymicrobial infection was encountered in 19%, 31% and 29% for patients with GB stones, CBD stones and IHD stones respectively; frequency of mixed , aerobic and anaerobic infection was 7%, 12% and 9%.

In a study by Irfan Sattar et al (2009) 36% had positive bile culture with E. coli as commonest organism followed by Klebsiella, Pseudomonas and Staphylococcus aureus.

In a study by Pratik m Parikh et al (2015) E. coli (15.38%) was the most common organism isolated from bile cultures. Other organisms were Pseudomonas (3.85%), Klebsiella (2.56%), coagulase negative Staphylococcus (1.28%) and Staphylococcus viridans (1.28%).

Thus we see that the pattern of the biliary bacterial flora remains fairly constant as regards the presence of E.coli.

Other organisms commonly found were the different enterococci like klebsiella , proteus , pseudomonas , proteus but the percentage of the various Enterococci in various studies varied.

This was followed by other relatively rare bacteria like streptococci, staphylococci, anaerobic bacteria etc again their percentage varying in different studies.

Mixed aerobic culture was found in 9 cases (19.50%) of these, E. coli and Klebsiella combination were 7(77.80%) as compared to E.coli and staphylococcus combination and E.coli and pseudomonas combination, which were present in one case each (11.10%) . Literatures available show that mixed coliform growths is maximum as compared to others, but figures to confirm this finding were not available.

In our study, 45(97.82%) of the cases had positive aerobic bactobilia whereas only 1 (2.17%) had anaerobic culture. According to Finegold (1977), anaerobes were recovered from 1% to 6% specimen in whom routine bile culture was done. Biliary tract infection involving anaerobes, apart from acute clostridial cholecystitis has been described infrequently in literature.

In our study, the relative lack of anaerobes in the bile culture can be attributed to absence of complex multiple biliary tract surgery, biliary enteric anastomosis and cases of acute clostridial cholecystitis. There is a definite relationship of positive bacterial culture and the age of the patient with biliary tract diseases

in our study it was 50% in the third decade 63% in the fourth decade, 80% in the fifth decade and 90 % in the sixth decade and early seventh decade. This rate in the positive culture with increasing age is statistically significant and almost similar as reported by Babu et al (1988) i.e. 37.5% in third decade, 50% in fourth decade, 68.7% in fifth, 70% in the sixth and 80% in seventh decade.

Likewise, its been reported by Truedson H et al (1983) that due to an increased occurrence of bacteria in gall bladder bile of elderly patients(>60 years), the use of intraperitoneal drain from a bacteriological point of view could thus be limited.

In our study, 8 patients were males and 62 were females. 75% of males reported positive cultures in bile, whereas 64.15% females reported positive bile cultures. Other studies show little difference between the presence of bacteria in bile in relation to sex.

Surgeries performed for the various biliary tract disease in our study were cholecystectomy in 82.90% cases, common bile duct bile exploration 2.9% cases, common bile duct exploration with cholecystectomy in 8.5% cases, and biliary enteric anastomosis in 5.70% cases. In the series of Willis et al (1978). 100% patients underwent elective cholecystectomy. In addition 13.3% patients required common bile duct exploration and 1.8% required sphincterotomy.

Fever is a good yardstick as regards assessment of infection in an individual. Of the 46 culture positive cases, 10 had pre- fever (21.6%), whereas fever was present in 2 cases (8.3%) of the 24 negative culture cases. Fever in the culture positive cases can be safely attributed to the biliary bacterial flora, leading to biliary bacteremia, whereas fever in the culture negative cases can be well explained by concomitant presence of other conditions e.g. Malaria, thrombophlebitis in patients on Intravenous drips etc.

Bile from the common bile duct was aspirated from 8 cases pre-operatively. Of these 5 cases showed positive culture 62.5% and the rest 3 cases showed negative culture report 37.5%. This when compared with the culture report of bile from the gall bladder, which shows 65 to 70% positive culture is almost identical in our series. In the study by Babu et al, (1988) the incidence of colonization of gallbladder and Common bile duct bile

was found to be significantly similar (41% to 42%). The segmental colonization, as reported by Willis et al (1978) ranged from 11.8% to 70%. The study by Bapat RD et al (1996) revealed analogous bacterial cultures from gall bladder, common bile duct and duodenum to be 63.2% and from gall bladder, common bile duct, nose, throat and urine 23.2%. It was also found that in all the patients identical bacterial species were isolated from all Segments. This finding strongly suggested that the organism infecting biliary tract were blood borne. Also, in our series, number of cases with positive common bile duct bile and negative gall bladder bile was only one. Similarly, number of cases with positive bladder bile and negative common bile duct bile was only two. These findings further give credence to the fact that incidence of bacterial colonization of gall bladder and common bile duct are almost identical.

As regards the types of bacteria found in the positive cultures from CBD Were E.coli 4 (40%), Proteus 2 (20%) and mixed aerobic 2 (40%). This does not commensurate with the findings of the all bladder bile culture in our study i.e. E. coli (52%), Klebsiella (17%), Proteus (7%), mixed aerobic (20%). No anaerobic organisms or staphylococci were found in the Common bile duct cultures, whereas they were present in 2% Cases of the gall bladder bile cultures. Virtually all series report high incidence of biliary flora when common bile duct stones are present, bacterial rate with jaundice. Also, wound infection rate has been found to be much higher when common bile duct was explored. Willis et al (1978) reports positive culture in 42.1% patients -with jaundice• Keighley et al (1975) reported 65% incidence of biliary sepsis in patients with jaundice at operation. He also found out that pathogens were much more common in presence of calculi (90%), than in patients with malignant obstruction (25%).

In our study, number of jaundiced patients was 8, and in all of them common bile duct exploration was done. Bile was found to be positive in 5 out of 8 jaundiced patients 62.5%. This figure relates well with the above findings, though the pattern of positive culture is certainly not found uniform in most of the studies.

Flemma et al (1967) reported an incidence of wound infection of 70% when presence of bacteria in bile was detected at time of operation. Truedson H et al (1983) noted that growth of bacteria analogous to those found in the drainage fluid of patients who underwent cholecystectomy was observed in gall bladder bile (5% of the patients), in pre-Operative skin culture (12%) and in the drain wound secretion (14%)• In all, but one patient, the organisms cultured from the wound were same as found in the bile. This finding lends credence to the fact that high incidence of wound sepsis and septicemia, particularly in jaundiced patients are associated with operation of biliary tract.

However, in our series, wound infection was found to be positive in only 22 out of the 46 bile positive cases (48%). Culture from the site of infection was not carried out, so the organisms responsible could not be made out.

Conclusion

We concluded with utmost assertion that taking bile cultures in all patients undergoing biliary tract surgery is mandatory , going by the high overall rate of septic complications occurring almost entirely in cases with biliary bacteremia , which is , as can be said from our study due to internal contamination from bile at the time of operation.

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