

Comparative Study of Bacterial Flora of Bilein Biliary Diseases and Normal Gall Bladder

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Abstract: *Introduction:* Gall bladder disease has a wide geographical variation being common in developed country. The bile tract probably does not harbor bacteria at most times in normal individuals. Bile in gall bladder or bile duct in the absence of biliary diseases normally sterile but in presence of any gall bladder stone and obstruction the prevalence of bactibillia increases. *Material and Methods:* The retrospective study was done in total 100 cases among these 100 cases 50 were having biliary or gall bladder diseases and comprised the study group while the remaining 50 were having other gastrointestinal diseases having no involvement of gall bladder and comprised the control group of study. *Result:* In this study of 100 case, 50 study group cases have positive microbial flora and in 50 control group cases only 3 case have positive microbial flora. Out of the positive 23 patients in study group, 21 cases have cholecystitis or cholelithiasis and from the control group, 3 positive cases have gram negative flora. *Conclusion:* Detection of microbial isolation in bile proved to be helpful tool in planning and antibiotic therapy for patient that helped in reducing hospital stay and avoid any major complication/expiry. Normal biliary tract infected by gram negative infection and our all 3 case positive in control group is gram negative.

Keywords: Bacterial Flora, Bilein Biliary Diseases And Normal Gall Bladder

1. Introduction

Gall bladder disease has a wide geographical variation being common in developed country. The bile tract probably does not harbor bacteria at most times in normal individuals. Bile in gall bladder or bile duct in the absence of biliary diseases normally sterile but in presence of any gall bladder stone and obstruction the prevalence of bactibillia increases. The condition necessary for the development of infection of biliary tract or for the production of infected bile are not in general known. It is agreed that infection regularly follow certain septicemic illness. The percentage of positive gallbladder bile culture among positive gall bladder bile culture among patient with symptomatic gall stone and chronic cholelithiasis range from 11% to 30. The majority of evidence favour an ascending route via duodenum as the main source of bacteria in bile.

Gram negative aerobes are organism most frequently isolated from the normal bile. In this e. coli and Klebsiella is most common and pseudomonas and enterobacter are most resistant.

However in various Gall Bladder disorders such as patient with acute and chronic cholecystitis yields organisms in 30%-50% of patients. Positive culture is common in acute rather than in chronic diseases and in those with obstructed as opposed to patent cystic duct. The incidence of positive culture in bile duct is greatest (75%-100%) when obstruction is caused by calculus and stenosis of surgical anastomosis.

In the present study as an attempt is being made to study and analyze the microbial flora of bile in patients with biliary disease in comparison to normal gall bladder.

2. Material and Methods

The present study was conducted in department of general surgery, G.G. Hospital Jamnagar, a total 100 patients were admitted in during period of June 2018- July 2019.

The retrospective study was done in total 100 cases among these 100 cases 50 were having biliary or gall bladder diseases and comprised the study group while the remaining 50 were having other gastrointestinal diseases having no involvement of gall bladder and comprised the control group of study.

Inclusion criteria

- 1) Age 18 year or above

Exclusion criteria

- 1) Cases with empyema and gangrene of gall bladder
- 2) Cases with evidence of Systemic Inflammatory Response Syndrome (SIRS) or sepsis
- 3) Patient having any prior use of antibiotics upto 3 month.

2.1 Method

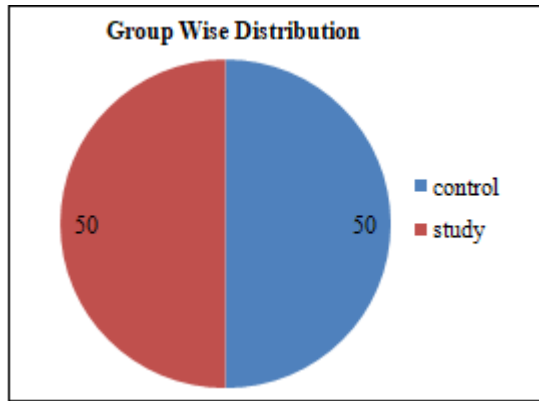
During operation, bile for culture was aspirated and collected from gall bladder by using a sterile syringe with 26 gauge needle under full aseptic precautions. Bile from normal gall bladder was aspirated during exploratory laparotomy 6ml of bile has been collected and was transferred to following containers:

- 2ml of bile was transferred on 0.1% thioglycollate or robertson's cooked meat media and was sent to department of microbiology, SGPGI Lucknow for anaerobic culture
- Remaining 4 ml of bile in culture bottle was transferred immediately into brain heart infusion (BHI) broth and was sent to department of microbiology Era's Lucknow medical college and hospital lucknow for aerobic culture and sensitivity.

3. Results

1) Group wise Distribution

S.No	Group	Description	No of subject	Percentage
1	Control	Patient undergoing gastrointestinal surgery except biliary or gall bladder diseases	50	50
2	Study	Patient who had undergone surgery for biliary diseases	50	50

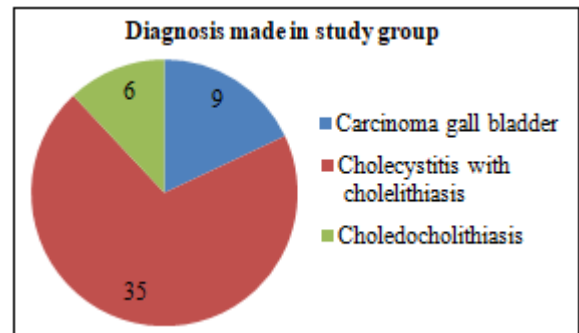
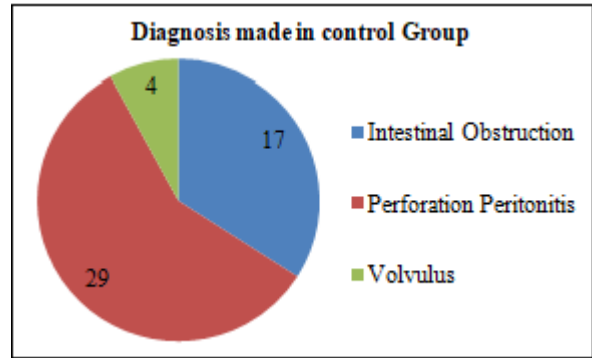


A total of 100 subjects were enrolled in the present study to study the microbial flora of bile in patient with biliary diseases in comparison to normal gall bladder and to study the antibiotics sensitivity pattern of different types of bacteria obtained. According to the inclusion criteria they were grouped under two groups.

A total of 50(50%) subjects who had been operated upon for gastrointestinal diseases apart from those affecting gall bladder and biliary tract comprised the control group of the study while the remaining 50(50%) subject who had been operated upon for biliary tract or gall bladder diseases comprised study group

2) Diagnosis

S.No	Diagnosis	Number of Subject	Percentage
Control Group			
1	Intestinal Obstruction	17	34
2	Perforation Peritonitis	29	58
3	Volvulus	4	8
Study Group			
1	Carcinoma gall bladder	9	18
2	Cholecystitis with cholelithiasis	35	70
3	Choledocholithiasis	6	12



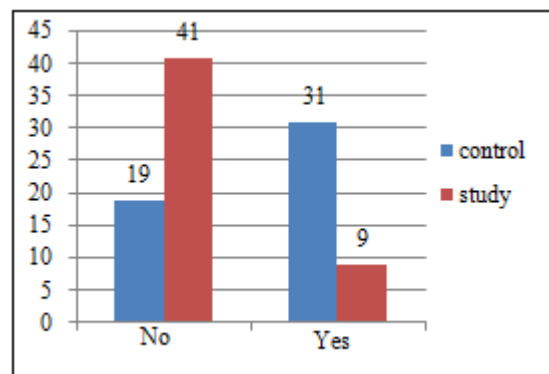
Among control subject, Perforation peritonitis (n=29; 58%) was the most common finding whereas amongst study subject cholecystitis with cholelithiasis was the most common finding (n=35; 70%)

There were 17(34%) cases of intestinal obstruction and 4(8%) cases of volvulus amongst controls.

There were 9(18%) cases of carcinoma gall bladder and 6(12%) cases of choledocholithiasis amongst study subject.

3) Associated Illness

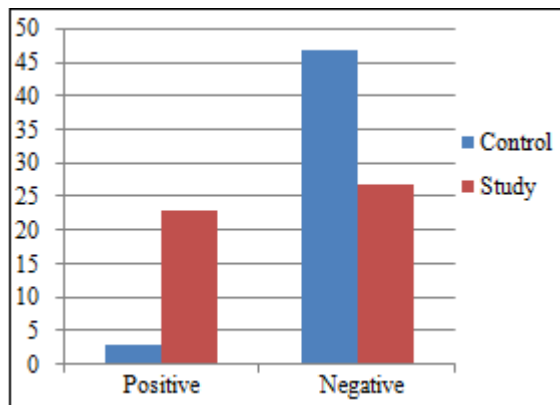
S.No	Associated illness	Control (n=50)		Study (n=50)	
		No	%	No	%
1.	Yes	31	62	9	18
2.	No	19	38	41	82



Majority of subject (62%) in control group had associated illness where in study group only 9(18%) subject had associated illness. In study group, 4 patient had diabetes mellitus, 4 hbsag positivity and one had HCV positivity where in control group 1 patient had Diabetes mellitus 1 had hydrocele 12 had tuberculous and 17 had typhoid fever. Statistically, there was a significant difference between two groups as regards presence of associated illness

4) Microbial Positivity

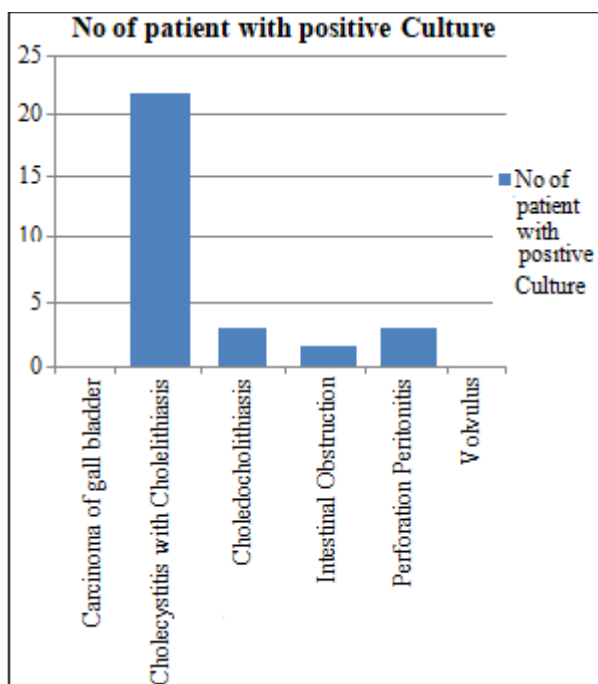
S.No	Positivity	Control (n=50)		Study (n=50)	
		No	%	No	%
1.	Positive	3	6	23	46
2.	Negative	47	94	27	54



In control group, 3(6%) of the sample were found to be positive where in study group, 23(46%) of the sample were found positive on comparing the data statistically, the incidence of microbial positivity was found to be significantly higher in study group as compared to control Group

5) Diagnosis and Microbial Flora

S.No	Diagnosis	Total No of patients	No of patient with positive Culture	Percentage
1.	Carcinoma of gall bladder	9	0	0
2.	Cholecystitis with Cholelithiasis	35	21	60
3.	Choledocholithiasis	6	2	33.3
4.	Intestinal Obstruction	17	1	5.88
5.	Perforation Peritonitis	29	2	6.9
6.	Volvulus	4	0	0



None of the carcinoma of gall bladder cases in study group and volvulus cases in control group had a positive culture finding. Maximum positivity was observed in cholecystitis with cholelithiasis group (60%) while Intestinal Obstruction and perforation Peritonitis indicated a positivity of 5.9% and 6.9% respectively. Choledocholithiasis Subjects had a positivity of 33.3%.

4. Discussion

The biliary tract probably does not harbor bacteria at most times in normal individuals. It seems quite unlikely that there could be the same sort of sparse indigenous flora that is seen in the duodenum since complete biliary obstruction is rarely associated with bacterial cholangitis

Bacterial infection of bile duct might play a critical role in the development of brown gall stone, while stones may format a back ground for bacteria development of some cases. Prompt Administration of the appropriate antibiotics in crucial in the management of the biliary tract infection and antimicrobial treatment is commonly administered Pre or post Operatively and often the bacterial growth.

Although normal bile does not contain any microbial flora but in presence of any gall bladder stones and obstruction the prevalence of bactibillia increase. The changed situation owing to changed status of gall bladder is responsible for the microbial growth in the bile has been supported by many workers. In general the bile does not contain any gram positive bacteria in normal state, microbial presence if any in normal gall bladder is marked by the Presence of Gram Negative.

In present study, a total of 100 patients were enrolled. The control group comprised of 50 patients who were undergoing gastrointestinal surgery except for biliary or gallbladder disease and study group comprised of patients who were undergoing surgery for biliary diseases. Though the ideal situation might have been to selected the patients without any gastrointestinal diseases as it might assumed that gastrointestinal disease of any kind might have any interactive role with gall bladder and bile. However the ethical issues do not allow choosing normal healthy human beings for bile collection. The collection of bile can be done only during a procedure involving an operative procedure no matter whether laproscopic or open. However we have taken care not to include any patient having such diseases that might affect the quality of bile being sampled.

In study group, cholecystitis with cholelithiasis was the commonest diagnosis (70%) followed by carcinoma of gall bladder (18%) and choledocholithiasis (12%). More than 95% of biliary tract diseases are attributable to cholelithiasis. Gallstones afflict 10-20% of the adult population in developed countries including united states. Though the prevalence of carcinoma of gall bladder is relatively low, ranging from 1.2 per 100000 population to 7.5 per 100000 in different part of world, the higher proportion in present study could attributed to the fact that carcinoma gall bladder is disease for which specialized care is required. Ours is a tertiary care teaching hospital with all facilities available for treatment of carcinoma gall bladder.

On this count, the relatively higher proportion of carcinoma gall bladder patients comprising the study group could be explained.

The associated illnesses in the control group were representative of the respective diagnosis; perforation peritonitis was the most common diagnosis in the control group which often has tuberculosis or typhoid fever as etiology. In study group, associated illnesses were diabetes mellitus in 4 patients and HbsAg positivity in another 4, while 1 patient had HCV positivity. Diabetes mellitus is considered to be a lifestyle disorder; the role of changing lifestyle and dietary habits has been reported in literature. Lu et al. (1990) reported a significant association of cholelithiasis with diabetes mellitus. In their study, they encountered patient having HbsAg positivity to be suffering from cholelithiasis, however they did not find the association between two to be significant.

In present study out of 50 samples in each groups microbial positivity in bile was seen in 3(6%) of control group subjects having no gall bladder related diseases as compared to study group where this proportion was 23/50(46%)

In general, the bile does not contain any gram positive bacteria in normal state, microbial presence if any in normal gall bladder is marked by the presence of gram negative bacteria. In present study too all the three cases having microbial growth in control group were found to be having gram negative bacteria. In two of these cases E.Coli was found while in the remaining one strain of salmonella species were recovered. The last case was having typhoid fever which ultimately resulted in perforation peritonitis

Al harbi et al. obtained bile sample from 112 patients undergoing elective laparoscopic cholecystectomy for gallstones and found twenty eight of these bile culture (25%) were positive, four of which contained more than one organism. However in present study, 23 out of 50 (46%) bile specimens were found to be positive for microflora.

Csendes et al. studied the number of bacterial colonies and the number of pyocytes per ml of choledochal bile. One hundred patients with symptomatic gallstones, 42 patients with common bile duct stones without cholangitis, 24 patient with common duct stones and acute cholangitis, and 42 controls were included in the study. The investigators detected no bacteria in the gallbladder bile of the controls. However in present study there were three controls in whom microbial flora was seen to be present.

However in the study of Capoor et al from a total of 104 bile samples bacteria were isolated in 37 samples (35.6%). The results obtained in the present study are close to the finding of Capoor et al though the relatively higher incidence of positivity (46%) is still than that quoted in literature and needs a further exploration. The findings are close to a study by Sharma et al. who studied a total of 390 patients in three groups gallbladder carcinoma 65(17%), cholelithiasis 125(32%) and control groups 200(51%). they found 116(30%) patient had culture positive bile. Significantly higher number of patient with gallbladder carcinoma 40(65%) had culture positive bile as compared to

cholelithiasis 52(42%) and control 24(12%). In present study, the culture positivity among cases was found to be 46% which is close to the 42% as reported by Sharma. The incidence of culture positivity in controls in present study was much lower (6%) than that reported by Sharma et al, However, out of 9 cases of carcinoma of gall bladder non was found to be culture positive in present study. These differences in literature suggest that though the microflora in controls is generally low the same varies depending upon nature and extent of gallbladder diseases.

In control group out of three sample with microbial positivity 1(2%) had E.coli alone E coli in combination with bacteriodes is 1(2%) and 1(2%) had salmonella species. In study group too E coli alone was most common microbial present in the bile (n=9; 18%) or in combination with bacteriodes spp (n=1; 2%) Bacteriodes sp. Was the next most common microbial, either alone (n=3; 6%) or in combination (n=1; 2%) No fungal infection observed in any case. Anaerobes are infrequent cause of biliary infections and are usually associated with chronic cholecystitis.

Amongst various antibiotics assessed for sensitivity for aerobes imipenem showed the maximum sensitivity (100%) followed by Amoxicillin+Clavulanic Acid and ceftriaxone (52.13%). Among other antibiotics showing sensitivity were Piperacillin+Tazobactam, amikacin, gentamycin, levofloxacin and ampicillin+sulbactam. All the other antibiotics had very high to absolute resistance

5. Conclusion

On the basis of observations made during the course of present study, the following conclusions have been drawn:

- 1) Cholecystitis with cholelithiasis was the commonest gall bladder disease encountered in the present study, affecting around three-fourth of the patients.
- 2) Females were more commonly affected by gall bladder disease compared to males.
- 3) Maximum numbers of patients with gall bladder disease were in their forties.
- 4) Only 9(18%) patients with gall bladder disease had any associated illness- Diabetes mellitus and HBsAg positivity contributed to 8% each.
- 5) Biochemical and hematological investigations did not reveal any significant change.
- 6) Laparoscopic cholecystectomy was the procedure of choice in majority of study group cases.
- 7) No significant information in terms of change in colour or consistency of bile was obtained.
- 8) The microbial positivity was 46% in study group and 6% in control group.
- 9) Polymicrobial positivity was encountered in 1 cases of study group having co-infection of E. coli and Bacteriodes species.
- 10) No gram positive strain was isolated.
- 11) E.coli was the most common species either alone or in combination with bacteriodes species.
- 12) None of the patients with carcinoma Gall Bladder had a positive culture finding.
- 13) Maximum positivity was observed in patients of cholecystitis and cholelithiasis followed by choledocholithiasis.

- 14) Amongst controls, patients of perforation peritonitis had maximum culture positivity(6.9%) while the patient of volvulus did not show any positive finding.
- 15) Patient in higher age groups (>50 years) had significantly higher positivity as compared to patients in lower age groups (<50 years)
- 16) For aerobes, imipenem showed the maximum sensitivity (100%), followed by amoxicillin+clavunic acid and Ceftriaxone (52.13%). Among other antibiotics showing sensitivity were piperacillin+ tazobectum, amikacin, gentamycin, levofloxacin and ampicillin+sulbactam . All the other antibiotics had very high to absolute resistance
- 17) For anaerobes, Imipenem+Cisplatin and metronidazole showed 100% sensitivity followed by clindmycin (60%) and chloramphenicol (20%). Anaerobes were fully resistant against all the other antibiotics used for assessment.
- 18) Only six Cases had hospital stay of >10 days who had undergone open choledocholithotomy and two of these was culture positive. In all these open procedure was done
- 19) During the course of study, one expiry took place. It was a patient of carcinoma pf gall bladder, this patient too did not have a culture positivity

Detection of microbial isolates in the bile proved to be a helpful tool. In planning an antibiotics therapy for the patients that helped in reducing the hospital stay and avoiding any major complication/expiry

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