

ORIGINAL RESEARCH

Study of the spectra of organisms in relation to biochemical analysis of gall bladder calculi

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ABSTRACT

Background: Gallbladder stores and concentrates bile. Various factors influence the formation of gallstones. Bile infection is one of the important factors amongst all the other factors. There is a specific correlation between spectra of organisms in bile and biochemical composition of gallbladder calculi. **Methods:** Present study was conducted to assess the same where we studied the microbiology of bile and analysed the composition of gallstones by specific set techniques. **Result:** Infectious factor plays an important role in formation of pigment gallstones as compared to cholesterol and mixed stones most common bacteria being *Escherichia coli*. **Conclusion:** Moynihan's aphorism that "gallstone is a tombstone erected in the memory of the organism within it" is true even today and can be concluded from our study too, Rate of bile infection increases with increasing age. Hence collecting bile samples for culture study and determining biochemical composition of gallbladder calculi should be emphasised in our routine surgical practice.

Keywords: Gallbladder, Gallstones, bile, biochemical, microbiology

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INTRODUCTION

Gallstones are classified into cholesterol, pigment and mixed stones. Stones composed mostly of cholesterol account for 80% to 90% of patients undergoing cholecystectomy in western countries. The pathogenesis of gallstones is multifactorial. It varies according to the type of gallstones. Mixed gallstones are frequently associated with cholecystitis. In about half of the cases bacteria can be cultured from the gallbladder bile.[1] Infective factor seems to be the major cause of formation of gallstones. Moynihan's aphorism that "gallstone is a tomb stone erected in the memory of the organism within it" is true today.[2]

Human bile is normally sterile. However in biliary tract obstruction and in cholelithiasis bacteria may gain access to the biliary tract either through the papilla of Vater or the portal circulation and

subsequently lead to biliary tract infection.[3] In infected bile, the typical pathogens are gram-negative enteric aerobes such as *Escherichia coli*, *Klebsiella* species and *Proteus* species, while *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Enterococcus faecalis* are less commonly cultured.[4] Evidence in favour of infection also includes isolation of *Bacterium typhosum* and *Streptococcus* from the gallbladder bile and centre of the gallstones. Slow growing *Actinomyces* also have been recovered from the bile. These organisms reach the gall bladder via blood stream from infective focus elsewhere in the body or via lymphatics.[5] *Salmonella enteric* serovar Typhi can colonize the gallbladder and persist in asymptomatic carrier state that is frequently associated with presence of gallstones. Bile should be cultured for more accurate picture.[4] Bile infection by *Escherichia coli* precedes rather than follows brown

stone formation. Infection together with bile stasis is main factor in the pathogenesis of brown pigment stones.[6] Bacteria recently were identified in majority of pigment stones and in pigment portion of composite stones therefore bacterial infection has been considered as a primary factor, not only in pathogenesis of brown stones but also in formation of black pigment stones.[5]

MATERIALS AND METHODS

Source of data: Patients admitted to our hospital with the diagnosis of gallstone disease for open and laparoscopic cholecystectomy were taken for this observational study. 50 cases with calculous cholecystitis or chronic cholelithiasis in all the age groups were studied. Informed consent of all the patients included in the study was obtained and permission from ethics committee of institution was taken. Patients were informed about aims and procedures of the study. Detailed history and clinical examination of each and every patient was done. No prophylactic antibiotic was given to patients prior to surgery. Appropriate dosage of antibiotics was administered par table after collection of bile.

Bile Culture: 5-10ml of bile sample was aspirated with sterile syringe and 3-5ml sample of bile in sterile container was sent to the department of microbiology. The aerobic culture of bile sample was put on blood agar and Mac Conkey agar. The plates were incubated at 37 degrees Celsius for 24-48 hrs. The growth obtained was identified on the basis of colony morphology and standard biochemical tests.

Biochemical analysis of gallstones: Devised by Oser[7]: Gallstones were collected in sterile container immediately after cholecystectomy and were washed and sent to the department of biochemistry for

qualitative biochemical analysis of cholesterol, pigment and mixed components as per the following protocol:

Procedure: The stone was ground and dissolved in ether. Ether extract was evaporated and residue was divided into two parts for confirming the presence of:

1. Cholesterol
2. Bile pigments

Cholesterol was analyzed qualitatively as per the following:

- To first residue chloroform was added and this was followed by addition of sulphuric acid. Appearance of brownish green color indicated the presence of cholesterol.
- After addition of chloroform, sulphuric acid and ferric chloride were added. Appearance of pink color indicated the presence of cholesterol.
- After addition of chloroform, acetic anhydride and sulphuric acid were added in ratio of 10ml: 0.1ml, appearance of green color indicated the presence of cholesterol.

Bile pigments were analyzed qualitatively as per the following:

- N/10 hydrochloric acid was added in the second part of residue
- It was filtered
- Filter paper was washed with water and dried
- 5ml chloroform was added and again filtered
- Red/yellow color of filtrate indicated the presence of bilirubin
- Appearance of green color on addition of hot alcohol on filter paper indicated the presence of biliverdin

RESULTS

Table – 1 Incidence of bile infection

Age (in years)	Bile Culture positivity	Percentage (out of 50)
21-30	1	2%
31-40	2	4%
41-50	3	6%
51-60	6	12%

Bile culture was positive in 12 cases, out of which 9 cases were females, 3 were males. Majority of positive cases were seen in the age group of 51-60 years and in females.

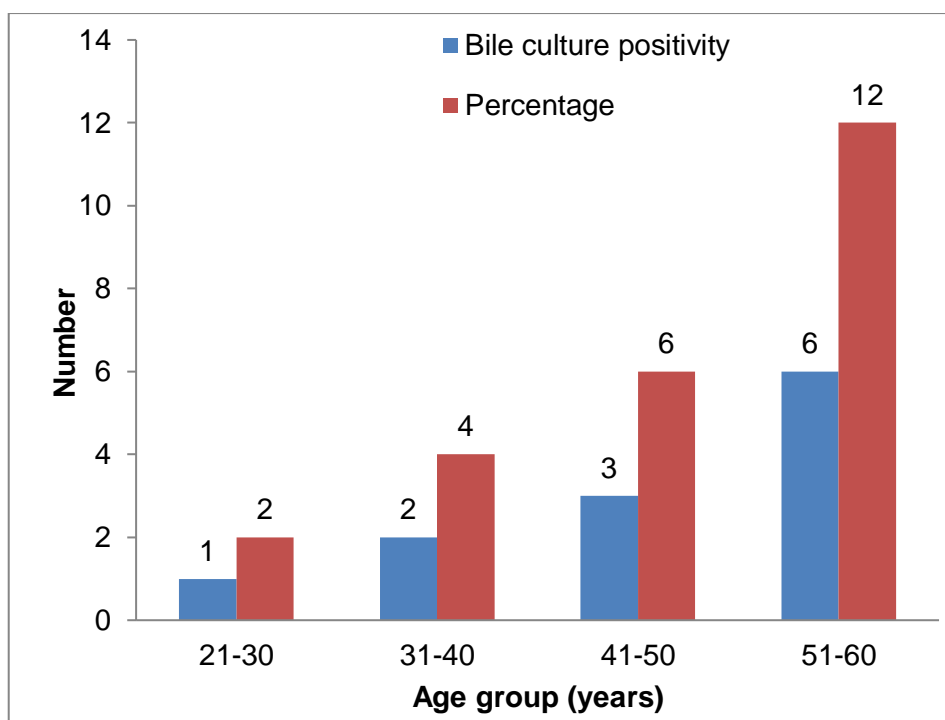


Fig. 1: Incidence of bile infection

Table –2 Bile culture positivity in different type of gallstones

Gall stone	Cases	Bile culture positivity	Percentage
Cholesterol	26	1	3.8%
Pigment	22	11	50%
Mixed	2	0	0%

Bile culture was positive in 1 case of cholesterol gallstone disease. 11 cases in pigment gallstone disease showed bile culture positivity out of 22 cases. Culture positivity was not seen in mixed stones.

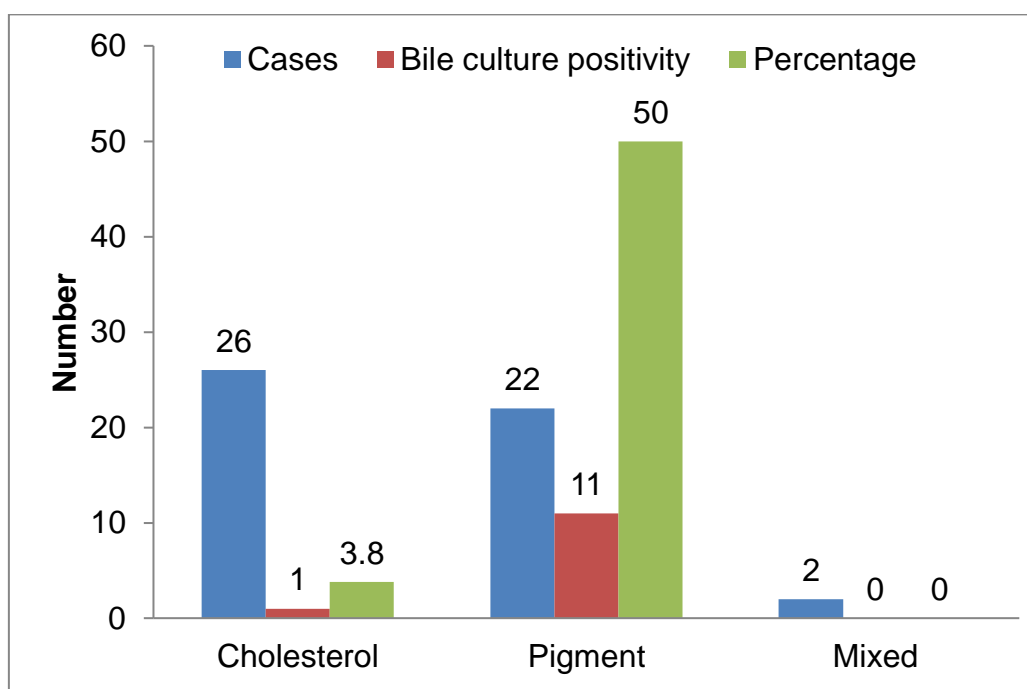


Fig. 2: Bile culture positivity in different type of gallstones

Table – 3 Common bacteria isolated from bile culture

Microorganisms	Bile culture positivity
Staphylococci aureus	2
Escherichia coli	6
Enterococcus faecalis	1
Klebsiella pneumoniae	1
Pseudomonas aeruginosa	2

Commonest bacteria isolated from bile were **Escherichia coli**.

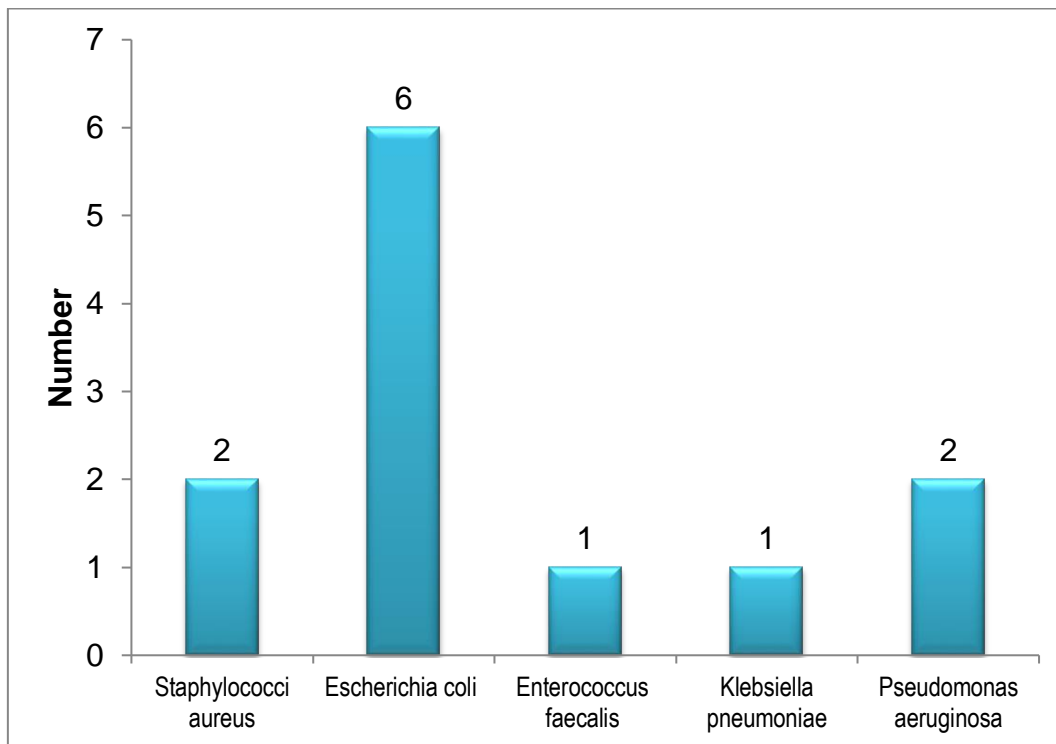


Fig. 3: Common bacteria isolated from bile culture



Fig. 4: Culture of Bile

Above black line: Bacterial growth of *Escherichia coli*
(Lactose fermenting pink colonies)

Below black line: Bacterial growth of *Pseudomonas aeruginosa* (Non lactose fermenting pale colonies)

DISCUSSION

This study was undertaken to study the spectra of organisms in relation to biochemical composition of gallbladder calculi. With the materials and methods devised bile infection and biochemical composition of gallstone was determined and relevant correlation between them was studied and compared to different studies as shown.

Bacteriology of bile and comparison of our study with existing studies:

In the majority of publications 25-35% patients undergoing biliary surgery were found to harbor bacteria in the bile. In his study Mr. R.G. Willis of Park hospital, Manchester bile of 76 patients

was cultured from gallbladder and 9 cases were positive for culture. Culture media used for bile samples were blood agar and Mac Conkey agar without salt, Deoxycholate citrate agar, cooked meat media. Common bacteria isolated from bile in our study were Escherichia coli. In R.G. Willis and Lawson study were also Escherichia coli.[8] They also showed that as age advances, so does the likelihood of culturing bacteria from the bile of biliary surgery cases. In our study majority of bile culture positive cases were seen in the higher age group range of 51-60 years (Table 3).Hence the findings of the R.G Willis and W.C Lawson study are consistent with our present study.

Table – 4 Comparison of bile culture positivity in our series with R.G. Willis series

	Cholelithiasis cases	Bile Bileculture positivity	Percentage
Our series	50	12	24%
R.G. Willis series	76	9	12%

In their series, R.G. Willis (Manchester) found bile culture positivity in 9 cases out of 76 cholelithiasis cases (site cultured gallbladder bile). In our series bile culture positivity was found in 12 cases out of 50 cases.

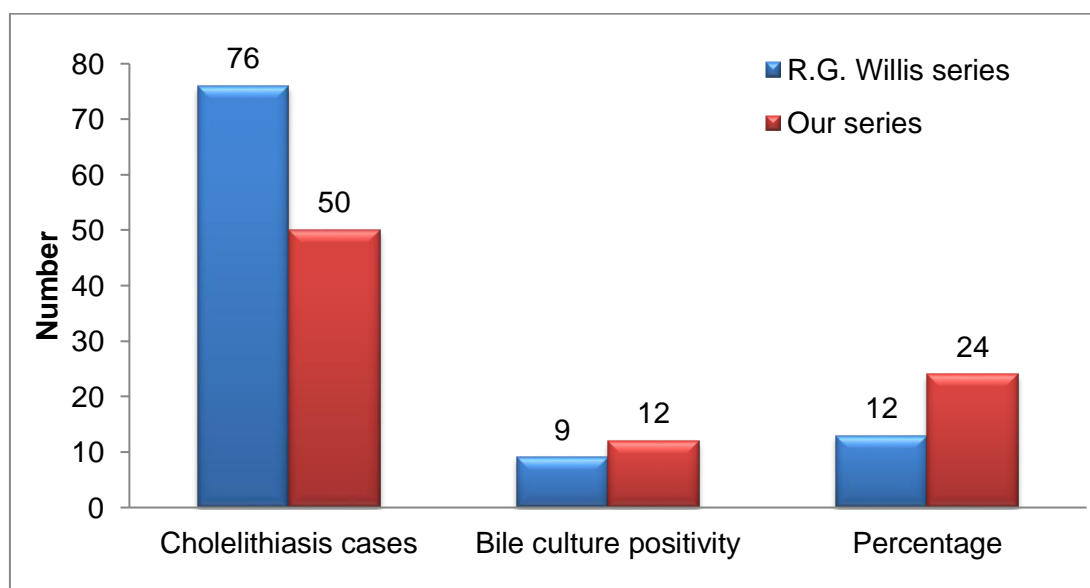


Fig. 5: Comparison of bile culture positivity in our series with R.G. Willis Series

In their study Lygia Stewart and J. Macleod Griffiss with 285 patients with symptomatic gall stone disease, 145 patients showed bile culture positivity. The percentage was 51%.[9] In our study out of 50 cases, 12 cases were bile culture positive. The percentage was 24%. Bacterial biliary tree colonization increases with age and is

more common with pigment stones. Not all bacterial species cause infectious manifestations. Patients with Escherichia coli and Klebsiella species commonly show infectious manifestations. This is also similar in comparison and hence consistent with our present study.

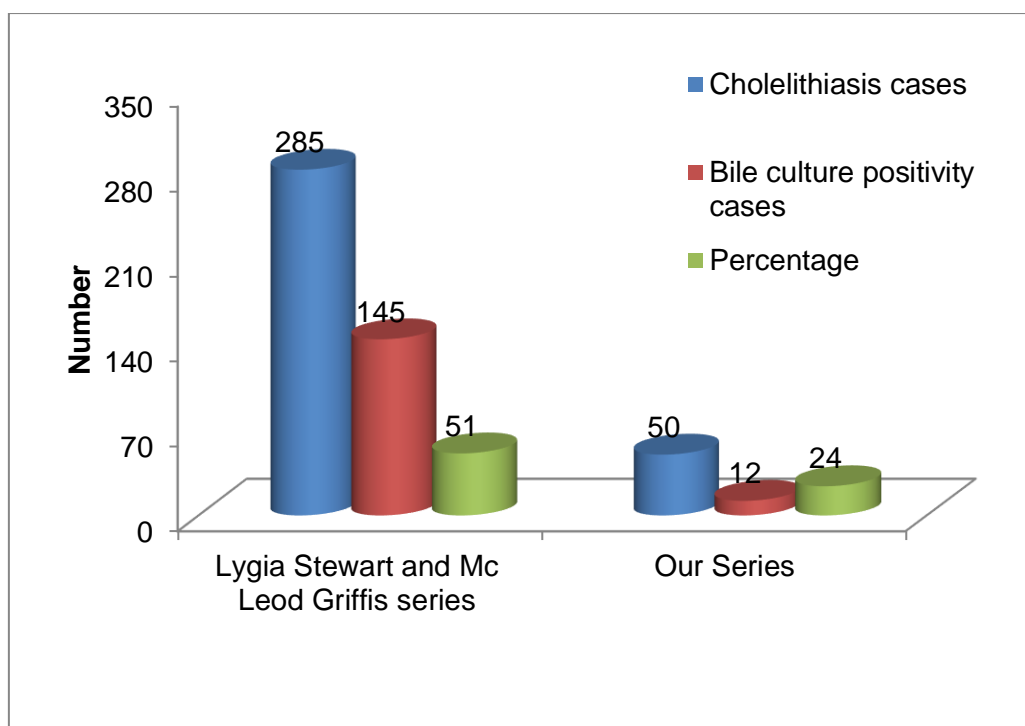


Fig. 6: Bacteriology of bile in percentage in cholecystectomy cases

Howards S. Kaufman from Department of Surgery, the John Hopkins Medical Institution studied 65 patients undergoing cholecystectomy for cholelithiasis, bile was cultured and stone cholesterol content was measured. Gall bladder stones in 65 patients were identified as cholesterol gallstones in 46 patients (71%), pigment gallstones in 19 (29%) patients. Bile cultures were positive in 13% of patients with cholesterol stones, in 14% of those with black pigment stones, and in all of the patients with brown pigment stones. They concluded that black and brown pigment gallstones have different pathogenic mechanisms and that bacterial infection is important only in the formation of brown pigment stones.[10]

In our series out of 50 patients who underwent cholecystectomy for cholelithiasis, 26 patients had cholesterol gallstones 22 patients had pigment gallstones and 2 patients had mixed gallstone and bile culture was positive in 1, 11 and 0 cases respectively with percentage of bile infection 3.8%, 50% and 0% respectively. Bacteria produce β -glucuronidase phospholipase enzyme which degrades the bile and causes pigment gallstone formation. Pigment gallstones contain calcium palmitate, calcium bilirubinate and conjugated bilirubin which are associated with bile infection. Hence infection plays an important role in pathogenesis of pigment stones more importantly brown pigment stones.

Table – 5 Bacteriology of bile in different type of stones

Stones	Howards S. Kauffman	Our series
Cholesterol	46 (6)	26 (1)
Pigment	19 (4)	2 (11)
Mixed	0 (0)	2(0)

In our series out of 26 cholesterol gallstones 1 case showed bile culture positivity and 11 cases out of 22 pigment gallstones showed positive bile culture. In Howards S. Kauffman study 6 cases in 46 cholesterol gallstone disease and 4 out of 19 pigment gallstone disease shows positive bile culture results.

CONCLUSION

Cholesterol is the major biochemical component in gallstones and plays a significant role in their formation. Infectious factor plays an important role in formation of pigment gallstones as compared to cholesterol and mixed stones most common bacteria being Escherichia coli as seen from different studies. Moynihan’s aphorism that “gallstone is a tombstone

erected in the memory of the organism within it” is true even today and can be concluded from our study too, Rate of bile infection increases with increasing age. Hence collecting bile samples for culture study and determining biochemical composition of gallbladder calculi should be emphasised in our routine surgical practice.

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