

**A Retrospective Study on Culture of Gall Stones Associated with Gall Stone Diseases & its Chemical Analysis.**Ayesha Farheen<sup>1</sup>, Najmussahar Kudchi<sup>2</sup>, Mohd Shahnawaz Ahmed<sup>3</sup><sup>1</sup>Associate Professor, Department of Pathology, ESIC Medical College and Hospital Kalaburagi, Karnataka<sup>2</sup>Assistant Professor, Department of Pathology, ESIC Medical College and Hospital Kalaburagi, Karnataka<sup>3</sup>Senior Resident, Department of Pathology, ESIC Medical College and Hospital Kalaburagi, Karnataka

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**Abstract**

Interest in the formation and clinical management of gallstone disease dates back to ancient times as archaeological evidence suggests that members of the royal Egyptian family were afflicted with this disorder. Gallstone disease continues to be a major health problem in this country and throughout the world, with estimates indicating that it is on the rise. The development and worldwide application of laparoscopic cholecystectomy has once again focussed our attention on gallstone disease.

**Materials and Methods:** This retrospective study was carried out in the Department of Pathology, ESIC Medical College and Hospital Kalaburagi, Karnataka.

**Observation:** Most of the patients belong to the category of chronic cholecystitis (52%). In this group chronic cholestasis with gallstones only was the most common presentation (32%). As expected, the female cases are more in all types of cases especially in chronic calculus cholelithiasis.

**Conclusion:** Most of the organisms were isolated as single isolate. Poly isolate cases were associated with complicated cases. It is better documented in cases like empyema, which showed positive culture reports having klebsiella, E. coli as well as bactericides which is as an anaerobe.

**Keywords:** Gall Stones, Culture Analysis, Cholecystectomy.

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**Introduction**

Interest in the formation and clinical management of gallstone disease dates back to ancient times as archaeological evidence suggests that members of the royal Egyptian family were afflicted with this disorder. Gallstone disease continues to be a major health problem in this country and throughout the world, with estimates indicating that it is on the rise. The development and worldwide application of laparoscopic cholecystectomy has once again focused our attention on gallstone disease. As has been described by Welch (1980), microorganisms play an important role in the formation of gallstones. Moynihan (1905) described the gallstone as a 'tombstone created in the memory of dead bacteria'. The presence of bacteria in gallstones cultivated interest in the infective cause of gallstone formation, many studies have been performed to culture bacteria from different types of gallstones and to establish its relationship with etiology of gallstone disease. Positive reports have been obtained by Rosenon, Illingworth, Gupta et al, Cetta. They have demonstrated various aspects of this single disease.

Relationships have been established between mixed stones and bacterial colonisation of the stones, thereby establishing colonisation to be an important factor in the pathogenicity of gallstones. This prompted a study to be undertaken for analysis of the relationship between biochemical type of stone and gallstone culture. The sub-population with gallstone disease would be subjected to biochemical analysis and culture of gallstone. This assignment will bring forth not only the etiology but also help to administer empirical treatment to patients [1-5].

**Materials and Methods**

This retrospective study was carried out in the Department of Pathology, ESIC Medical College and Hospital Kalaburagi, Karnataka. An equal number of cases were taken up as controls who were admitted for diseases other than biliary tract disorders and underwent laparotomy for the same.

## Results

Table 1: Age Distribution

Ag	Female	Male	Total
20-19	02	01	03
30-19	04	01	05
40-49	14	06	20
50-59	08	05	13
60-69	05	04	09

Gallbladder and biliary tract diseases were found to be more precedent in the age of 40 - 49 i.e 40% of the total number of cases. There is a female dominance which gradually this down with advancing ap. Age groups in controls varied from 20

- 70 years. 35 out of 50 cases %if of the average age groups of 30 - 40 years. Controls consisted of parents with stone diseases other than gallstones and CBD stones.

Table 2: Sex Distribution

Ag	Female	Male	Total
20-19	02	01	03
30-19	04	01	05
40-49	14	06	20
50-59	08	05	13
60-69	05	04	09

As can be seen, there is remarkably increased number of female patients (66%) in the cases. Therefore, the controls were taken with a bias tabard the fairer sex.

Table 3: Body Weight

Cases (n=50)Weight	40-45	46-50	51-55	56-60	61-65
Female-33	01	01	08	09	14
Male-17	-	02	06	07	02

Most of the females in this study were above average weight but most of the males were of average weight 69.60% of females were overweight.

Table 4: Pathological Distribution of Cases

Type of Cases	No.	Percentage	Male	Female
Acute cholecystitis	15	30	5	10
Chronic cholecystitis with gallstones only	16	32	5	11
Chronic cholecystitis with gallstones CBD Stones	10	20	4	6
Empyema gallbladder	06	12	2	4
Hydrophs of gallbladder	01	2	9	1
Malignancy of gallbladder	02	4	1	1

Most of the patients belong to the category of chronic cholecystitis (52%). In this group chronic choleystitis with gallstones only was the most common presentation (32%). As expected, the female cases are more in all types of cases especially

in chronic calculus cloleystitis. The disparity in the ratio closes down in cases with chronic cholecystitis with CBD stones, empyema gallbladder and in malignancy of gallbladder. The number of hydrophs cases was one.

Table 5: Symptoms of Presentation

Symptoms	No. of Cases	Percentage
Dyspepsia	32	64
Pain abdomen	42	64
Pain abdomen with vomiting	22	64
Pain abdomen, vomiting, Jaundice	06	12
Pain, Jaundice, fever	04	08
Pain, vomiting, fever	10	20
Pain, Jaundice, fever with features of shock	00	00
Vomiting, Jaundice, no pain	02	04

Abdominal pain was the most common presentation.

Abdominal pain ranged from mild pain to severe pain

lasting 12 - 24 hours. Therefore, it was a symptom present in almost all cases of gallstone diseases. Malignancy cases did not present with pain. Dyspepsia was present in all chronic cholecystitis cases. It was present in other cases too. Jaundice was

a predominant feature in cases with CBD stones. But classical charcoti triad was present in 08% cases and Reynoldpentad was absent. Fever was also associated with other symptoms, it was never the chief complain.

**Table 6: Per-Operative Finding**

Finding	No. of Cases
Gallbladder distended	11
Gallbladder wall thickened	10
Pericholecystic adhesions	12
Palpable calculi in gallbladder	48
Palpable calculi in CBD	10
Dilated CBD	10
Gallbladder growth	02
Secondaries in liver	02
Enlarged lymph nodes	02

The cases found to have dilated gallbladder were 11 out of which 6 were empyema gallbladder cases. Gallbladder was thickened in 10 cases out of which 8 cases were chronic calculus cholecystitis and 2 were malignancy cases. Pericholecystic adhesions were present in 12 cases out of which 6 cases were acute cholecystitis, 4

chronic cholecystitis, 1 each of malignancy and empyema. 48 out of 50 cases had palpable stones and 10 out of 10 cases with CBD stones were palpable. A hard growth was felt in both cases of carcinoma gallbladder, in these two cases liver had secondary metastasis and lymph nodes were enlarged.

**Figure 7: Morphologic Findings of Gallbladder Post Operatively**

1. Size	4. Signs of inflammation present-37
Enlarge-11	5. Growths-02
Normal-35	6. Stone-50
Shriveled-04	
2. Contents	
Mucus-01	
Bile-43	
Pus-06	
3. Wall thickness	
Normal-40	
Thickened-10	

As expected, the gallbladders were enlarged in 6 cases of empyema. These 6 cases contained pus, whereas the only case of Hydrophs contained mucus, rest contained bile. Wall was thickened in 10 cases. Signs of inflammation were present in all

cases of acute cholecystitis (15), emphyema gallbladder (06), chronic calculus cholecystitis (10) and chronic calculus cholecystitis with CBD stones (6). Growth was seen in both cases of carcinoma.

**Figure 8: Positive Gallstone Culture & Physical Findings, TLC**

Clinical cases	Positive culture	Temp>37.3°C	Loterus	TLC>12000>20000	
1. Acute cholecystitis (n=15)	07	04	00	10	00
2. Chr. Calculus Cholecystitis (n=16)	08	00	00	04	00
3. Chr. Calculus cholecystitis with CBD stone (n=10)	02	04	06	00	04
4. Empyema (n=06)	04	06	03	04	00
5. Hydrophs (n=01)	00	00	01	00	00
6. Malignancy (n=02)	00	00	02	00	00
<b>Total</b>	21	14	12	18	04

Temperature was raised in 14/21 cases of positive stone culture. TLC>12,000 in 18/21 cases with positive culture, 12/21 cases had jaundice.

**Table 9: Type of Stone and Positive Culture**

Type of stone	No. of cases	Positive culture
1. Cholesterol	13	01
2. Mixed	33	20
3. Pigment	04	00

Mixed stones were the most common types of stones 20/21 positive cultures came from mixed stones. Only one cholesterol stone had positive culture. None of the pigment stones had positive culture.

**Table 10: Relationship of Positive Culture to no. of Stones**

No. of stones	No. of cases	Positive culture	Percentage
Solitary	15	02	13.33
Multiple	35	19	54.28

11 out of 15 solitary stones were cholesterol stones and they showed negative culture. Out of the 04 mixed stones 02 came out to be positive. 54.28% of multiple stones were positive as most of them were mixed stones (27/33).

**Table 11: Organisms Cultured from Gallstones**

Species	Total	Single isolate	Poly- Isolate
E.Coil	12	08	04
Kelbsiella	06	04	02
Streptococcus	03	00	03
Enterococcus	03	01	02
Bacteroides	01	00	00
Salmonella	01	01	00
Clostridia	00	00	00

The most common organism isolated was E.Coil (12/21) i.e. 57.14% Klebsiella follows E.Coil Streptococcus and Enterococcus were isolated in 03 cases each. Bactroides and Salmonella were present in 1 case each.

**Figure 12: Relationship of Biliary Tract Disease & Isolate**

Disease	E.Coil	Klebsiella	Streptococcus	Enterococcus	Bacteroides	Salmonella
Acute cholecystitis	04	02	01	02	00	00
Chronic cholecystitis with gallstone only	05	02	02	01	00	00
Chronic cholecystitis with gallstones CBD stones	01	01	00	00	00	01
Empyema gallbladder	02	01	00	00	01	00
Hydrophs of gallbladder	00	00	00	00	00	00
Malignancy of gallbladder	00	00	00	00	00	00

E.Coil was the most common isolate in acute cholecystitis as well as chronic calculus cholecystitis. It was the most common isolate in empyema gallbladder also. Bactroides was isolated in a case of empyema and salmonella in a case of chronic calculus cholecystitis with CBD stones.

### Discussion

This discussion is based on the data procured by studies conducted on patients having gallstone disease. The total number of cases amounted to 50. A similar number of cases who had other stone disorders were subjected to similar tests and were considered as controls. The number of females were 33 in comparison to males who were 17. Most of the patients belonged to the age group of 40 - 49 (i.e. 20 / 50). The ratio of Female : Male is 1.9:1. This has

been shown by other studies like the one by Colcock (3 : 1) in 1961 [6,7]. Gallstone disease has shown a linear relationship between increasing age and the prevalence of the disease. The proportion of cholesterol in bile to cholesterol saturation index rises with age. Moreover, with increasing age there is decreasing response of gallbladder to CCK - PZ. In females, due to progesterone, there is altered gallbladder motility, decreased emptying and increase in absolute and residual gallbladder volume. These effects cause stasis and stone formation. These effects are marked increased during pregnancy. Most cases in the study were elderly i.e. between 55 - 65. As earlier described decreasing gallbladder motility leading to stasis is the probable cause of gallstone disease in the elderly. 69.6% of female cases were Muslim. Dietary load of cholesterol is

more in these subgroups. This is probably the cause for increased number of cases having cholelithiasis, this highlighting the fact that increased cholesterol load plays an important role in cholelithiasis. Most of the cases in our study were suffering from chronic cholecystitis (52%). Studies by Gupta, Udupa and other studies also had a preponderance of chronic cholecystitis. It has been a little higher in other studies. But this is probably due to less number of cases in our study. Out of 52% of chronic cholecystitis 20% had CBD stones. Other groups have shown a relatively less number of cases. Calculi obstruction of CBD tallies with the results in literature (12.16%. Maingot's surg)[8]. Again, females are affected more than men. But the ratio of females to males comes down from 11:5 to 6:4 as one moves from chronic cholelithiasis to chronic cholelithiasis with CBD stones. Similar findings were seen with Udupa and Srinivas (1968). Of all the symptoms of presentation, pain abdomen stand out with highest percentage (84%). Pain abdomen ranged from tenderness in right upper quadrant of abdomen which was mild to severe lasting from 12 - 24 hrs. All cases of acute cholecystitis had pain abdomen which was severe in nature. Most of chronic cholecystitis cases had mild pain per abdomen. Malignancy cases did not have pain[9,10,11]. Dyspepsia was the second most common presentation (64%). It was mainly a feature of chronic cholecystitis cases. Many of them had received earlier treatment for APD without facing proper investigations. Jaundice was not a very common finding in our cases. Its probably because we were more mainly dealing with cholelithiasis and thereby neglecting other cases which can cause obstructive jaundice i.e. periampallry carcinoma or carcinoma head of pancreas. Jaundice cases were mainly confined to patients with common duct stones, malignancy. The cause of jaundice in case of common duct stone is understandably due to obstruction. The jaundice in carcinoma gallbladder was due to periportal lymph node enlargement encroaching upon the CBD and causing obstruction. Classical charcot's triad of pain, fever, jaundice was seen only in 4 out of 12 cases. Reynolls pentad was not seen at all. Of all the physical signs, right upper quadrant pain was present in 74% i.e 37 out of 50 cases. It was seen in all cases of acute cholecystitis (15), 10 cases of chronic cholecystitis with in the cholelithiasis, 6 cases of chronic choleystitis with CBD stones, all 6 cases of empyema gallbladder. Obstructive jaundice was seen in 12 cases out of these 2 cases were of carcinoma gallbladder. Classical murphy's sign (inspiratory arrest during deep palpation in the right quadrant was present in 10 out of 15 cases of acute cholecystitis. Palpable gallbladder was present in 11 cases, 6 were empyema gallbladder, 1 case of hydrophs, 2 cases of malignancy and 2 cases of acute cholecystitis. Palpable gallbladder in acute cholecystitis as due to

overlying omentum on the gallbladder. According to Principles of Surgery Schwartz, it should be 20%. But in our study, it was little less i.e. 3.33% (2/15). One case of carcinoma gallbladder had shifting dullness and hepatomegaly. 14 cases had fever, the some cases had tachycardia (>90/ min). As suggested in 'Maingots, abdominal surgery', infection does not play any role in pathogenesis of pigment stones, plays a lesser role in cholesterol stones, the study also has similar result. The overwhelming number of positive cases in mixed stones confirms the theory of infective pathology in the formation of mixed gallstones. In comparing the positive culture reports with the number of stones, the solitary stones had 13.33% and multiple stones had 54.28% positive culture. This is because, cholesterol stones are mostly solitary whereas mixed stones are multiple. The type of organism isolated were many. But the most common isolate was E.coli, accounting for 15 out of 21 positive culture reports i.e. 57.14%. It matching with the report by Rusenon, who isolated 7 E.coli from 33 positive cultures. It also matches with Illingworth who isolated 3 E.coli from 7 positive cultures and Rains et al who isolated 7 E.coli strains from 43 positive culture. Klebsiella was isolated in 6 out of 21 positive cultures i.e. 28.5%. It is similar to the study by Gupta et al who had found 3 out of 25 positive cultures, having klebsiella i.e 12%[12,13]. 3 isolates had enterococci and 3 had streptococci. It was similar to the study by lingworth and Rains who isolated to streptococci strains from their series. Gupta et al also showed 2 streptococci strains out of 25 positive cultures. Bacteroides was isolated in 1 case and so was salmonella isolated in 1 case. This further confirms the fact that E.coli plays a major role in the pathogenesis of gallstones. This is because it releases enzyme 13- glucuronidase which acute on bilirbin and converts it into its unconjugated insoluble form, there by encouraging stone formation (Maingot's abd. surg.). Isolation of streptococci varies with different reports in different periods of time[14-19].

### Conclusion

Diseases of the biliary tract was found to be more common in the age group of 40 - 49 (40%). There was a marked preponderance of female patients (66%). In higher age groups the Male : Female ratio narrowed down, which means that with increasing age the number of male patients increased. Most of the females were above average weight, but most of the males were of average body weight. Most of the organisms isolated were aerobic. E.coli being the most common isolate, (57.14%). Other organisms were also found like Kleibseilla, streptococci and enterococci, salmonella and bactroides were found only in 1 case each. Most of the organisms were isolated as single isolate. Poly isolate cases were associated with complicated cases. It is better

documented in cases like empyema, which showed positive culture reports having klebsiella, E.coli as well as bacteroides which is as an anaerobe. That probably is a reason for such a complication.

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