



A Study on Bacteriological Profile of Bile And Gallstone on Adult Patient of Cholelithiasis

KEYWORDS

Bile Stone, Gallstone, Bacteriological Profile

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ABSTRACT *Introduction-* Biliary calculus disease is one of the most common disorder affecting gastrointestinal tract. There has been marked rise in the incidence of gallstone disease in the west during past century. In India, it is more common in the North India than in South India. Similarly the incidence in eastern India is higher than western India. Evidence in favour of infection includes isolation of E.Coli, Klebsiella, streptococcus from the gallbladder and from the centre of gallstones. This was more common in brown stones rather than cholesterol or brown stones. Brown pigment gallstones occur as a result of infection. Bacteria are found with in calcium bilirubinate and protein matrix of brown pigment gallstones.

Material And Method- Patients admitted to Department of Surgery, Dr. Bhim Rao Ambedkar Medical Hospital, which is attached to Pt. J. N. M Medical college, Raipur with diagnosis of Gallstone disease for open and laproscopic cholecystectomy were taken for this prospective study from January 2013 to October 2014. Detailed history of every patient with special emphasis on pain in right hypochondrium, nausea, vomiting, fever, and jaundice were taken. Complete physical examination done, and the findings of each patient entered in the standard proforma. Culture was carried for aerobic gram positive and Gram negative bacteria only using recommended culture media. Histopathological examination of gallbladder was performed to confirm diagnosis.

Result- In present series the age maximum patient was found in age group of 41-50 years (28%). The youngest patient was 25 yr old and oldest was 68 yr old. Bile culture was positive in 12 female patient out of 37 and 3 male patient out of 13. Male patient comprises of 23.07 % and female of 32.4%. Gall stone culture was positive in 10 cases out of 50 (20%), of which maximum were in age group of 51-60, 4/11 (36.3%). The most common organism isolated from gallstone was E.coli. The histopathology report was suggestive of chronic cholecystitis in all the cases.

Conclusion- Culture of organism from gallstone need to be necessary and correlating of it with culture of micro-organism of that of bile.

INTRODUCTION

Biliary calculus disease is one of the most common disorder affecting gastrointestinal tract. There has been marked rise in the incidence of gallstone disease in the west during past century. In India, it is more common in the North India than in South India. Similarly the incidence in eastern India is higher than western India.

Incidence of gallstones increases with age. It is more common in female than male M:F = 1:4 and about 50% patient are asymptomatic.¹

The pathogenesis of gallstones is multifactorial. It varies according to gallstones. Primary gallstones can be divided into two major groups. First is pure gallstones contributing of 10% of gallstones. Second is mixed and combined gallstones which accounts for 90% of gall stones. Mixed gallstones frequently associated with cholecystitis. In about 50% of the cases bacteria can be cultured from the bile which in normal is sterile.³

Infective factor seem to be a major cause of formation of gallstones. Moynihan's aphorism "gall stone is a tomb erected in the memory of the organism within it", suggest bacteria inside gallstone have thought to be dead.² Stones spilled during laproscopic cholecystectomy causes variety of complications, intraperitoneal abscess.⁴ Experimental

studies have found implantation of gall stone in the peritoneal cavity of rat, rabbit, dog produces intraperitoneal abscess, adhesion, sinus which states that gallstone is dynamic viable home of bacteria rather than a tomb.

Evidence in favour of infection includes isolation of E.Coli, Klebsiella, streptococcus from the gallbladder and from the centre of gallstones. This was more common in brown stones rather than cholesterol or brown stones.³

These bacteria reaches the gallbladder via blood stream, from infective focus elsewhere in the body and lymphatics.

Brown pigment gallstones occur as a result of infection. Bacteria are found with in calcium bilirubinate and protein matrix of brown pigment gallstones.⁵

With the above background, the present study was conducted to see the bacteriological profile of bile and gallstone on adult patient of cholelithiasis in a tertiary care hospital of Raipur city (C.G.), India.

MATERIAL AND METHOD

Patients admitted to Department of Surgery, Dr. Bhim Rao Ambedkar Medical Hospital, which is attached to Pt. J. N. M Medical college, Raipur with diagnosis of Gallstone disease for open and laproscopic cholecystectomy were taken

for this prospective study from January 2013 to October 2014. Ethical considerations were met through intuitional ethical committee.

Inclusion criteria

Patient of age group 21-70years

All proved cases of gallstone disease who got admitted to the hospital for cholecystectomy.

Exclusion criteria

Acute cholecystitis
Acute acalculus cholecystitis
Empyema of gallbladder
Mucocoele of gallbladder
Gallstones with multiple common bile duct stones
Patient who refused surgery

A thorough clinical examination was done and the findings noted. The patients were subjected to an ultrasound examination of the abdomen and the presence of cholelithiasis confirmed by it associated acute or chronic cholecystitis was also noted. Patients who did not have cholelithiasis on USG were excluded from the study.

Detailed history of every patient with special emphasis on pain in right hypochondrium ,nausea ,vomiting ,fever ,and jaundice were taken.Complete physical examination done ,and the findings of each patient entered in the standard proforma.

Sample collection –

a) Bile – Bile is collected from excised gallbladder, put in sterile bottle transferred to laboratory within half hour, if delayed kept in freezer before transfer.

b) Gall stones –

After cholecystectomy gallbladder was cut open and largest stone was transferred to sterile culture bottle containing normal saline and immediately transferred to laboratory for stone culture. Retrieved stones, were divided into three groups based on the morphology of gallstones.

- i. Whitish to pale yellow as cholesterol stones.
- ii. Black to blackish brown as pigment stones.
- iii. Yellow brown and greenish white as mixed stones

In laboratory whole stone was immersed in 70% ethanol for 10 min for surface sterilization, it was then bisected (taking sterile precaution) and the core was scooped out for culture. Culture was carried for aerobic gram positive and Gram negative bacteria only using recommended culture media.

c) Histopathological examination of gallbladder was performed to confirm diagnosis.

Culture technique –

Bile – 3-4 drops of bile ,and nidus of gallstone was kept in Mac Conkey Media & Blood agar media .Both the media are incubated at 37 degree C for 18-24 hours .

Mac Conkey's media is differential media .It consist of peptone,lactose agar ,neutral red and taurocholate .By this media –lactose fermenter and non-lactose fermenter bacteria can be identified .Lactose fermenter shows pink colonies and non lactose fermenter shows colourless or pale colonies .

Blood agar – To identify staphylococci,streptococci,proteus

In blood agar Staph form oil paint like colonies in clusters, streptococci colonies are in chain .

If the grown bacteria is Staphylococci then Tube coagulase test is done to see wheather it is coagulase positive which are pathogenic,or negative which are non-pathogenic.

Data was compiled in MS-Excel and checked for its completeness and correctness. Then it was analyzed.

OBSERVATION AND RESULT

TABLE -1 AGE DISTRIBUTION OF CHOLELITHIASIS

Age group in years	Cholelithiasis patient	Percentage
21-30	7	14%
31-40	8	16%
41-50	14	28%
51-60	11	22%
61-70	10	20%
TOTAL	50	100%

In present series the age maximum patient was found in age group of 41-50 years (28%).The youngest patient was 25 yr old and oldest was 68 yr old . [Table-1]

TABLE 2:- GENDER WISE DISTRIBUTION OF CHOLELITHIASIS

SEX	Cholelithiasis patients	Percentage
Male	13	26%
Female	37	74%
Total	50	100

In this series females were maximum patient .Female to Male ratio was 2.9:1. [Table-2]

TABLE 3:- CLINICAL FEATURES OF CHOLELITHIASIS

Symptoms	No.of subjects	Percent
Rt Hypochondriac pain	48	96%
Vomiting	30	60%
Fever	9	18%
Flatulent dyspepsia	25	50%

Right hypochondriac pain was the main symptom in present series constituting 48 (96%) of the cases,Vomiting was the next common symptom found in 30(60%) of the cases. [Table-3]

TABLE 4 - Past history of treatment with Antibiotic course

Preoperative intervention	Male	Female	Total
Course of Antibiotic	5/13	20/37	25/50(50%)

In present study 25 patient had history of acute cholecystitis for which they were treated with course of antibiotic out of which 5 (38.4%) were male and 20(54%) were female. [Table-4]

TABLE 5 – TYPE OF GALLSTONE IN CHOLELITHIASIS BASED ON MORPHOLOGY

AGE	CASES	CHOLESTEROL STONES	PIGMENT STONES	MIXED STONES
21-30	5	3	2	-
31-40	9	3	6	-
41-50	18	6	10	2
51-60	10	7	3	-
61-70	8	2	6	-
TOTAL	50	21	27	2

Out of 50 cases of cholelithiasis, cholesterol gallstone were found in 21 cases,pigment gallstone were found in 27 cas-

es and 2 case were of mixed type of gallstones. Maximum cases had pigment type of gallstone. [Table-5]

TABLE 6:- INCIDENCE OF BILE INFECTION IN CHOLELITHIASIS

AGE IN YEARS	NO.OF SUBJECT	BILE CULTURE POSITIVE		TOOTAL	PERCENTAGE
		MALE	FEMALE		
21-30	7	0	1	1	14%
31-40	8	1	1	2	12.5%
41-50	14	0	5	5	35.7%
51-60	11	1	2	3	27.2%
61-70	10	1	3	4	40%
TOTAL	50	3(6%)	12(24%)	15	30%

Culture of Bile was positive in 15 cases out of 50 (30%),Majority of number of positive cases were in age group of 40-50 years. But the percentage of positivity was highest in age group of 61-70 years 4/10(40%).[Table-6]

TABLE 7- BILE CULTURE POSITIVITY IN MALE AND FEMALES

SEX	BILE CULTURE POSITIVITY
MALE	3/13(23.07%)
FEMALE	12/37(32.4%)

Bile culture was positive in 12 female patient out of 37 and 3 male patient out of 13. Male patient comprises of 23.07%and female of 32.4%. [Table-7]

TABLE 8- INCIDENCE OF POSITIVE BILE CULTURE IN DIFFERENT TYPE OF GALLSTONES

Type of gallstone	Total no.of cases	Positive bile culture	Percentage
Cholesterol	21	3	14.2%
Pigment	27	10	37.03%
Mixed	2	2	100%

Bile culture was positive in 10 cases of pigment stone out of 27(37.03%),3 cases of cholesterol stone out of 21(14.2%),and 2 cases of mixed type of stone out of 2.Maximum bile culture positive was in pigment stone. [Table-8]

TABLE 9- COMMON ORGANISM ISOLATED FROM BILE CULTURE

ORGANISM ISOLATED	Bile culture positivity	Percentage
E.Coli	10	66.6%
Klebsiella	2	13.3%
E.coli +Klebsiella	1	6.6%
Pseudomonas	1	6.6%
Acinobacter	1	6.6%
Bacteriods	0	0
Staphylococci	0	0
Streptococci	0	0

The most common isolated organism from bile culture was E.coli found in 66.6% followed by klebsiella which was present in 13.3% of cases. [Table-9]

TABLE -10: POLYMICROBIAL AND MONOMICROBIAL INFECTION OF BILE

Type of infection	No. of cases	Percentage
Polymicrobial	1/15	6.6%
Monomicrobial	14/15	93.3%

Out of 15 bile culture positive cases ,13 grew single type of organism,and in one more than one type of microorganism was found. [Table-10] .

TABLE 11- INCIDENCE OF GALLSTONE INFECTION

Age in years	Cholelithiasis cases	Positive gall-stone culture		Total	Percent-age
		Male	Female		
21-30	7	0	0	0	0
31-40	8	1	1	2	25%
41-50	14	0	2	2	14%
51-60	11	0	4	4	36.3%
61-70	10	1	1	2	20%
Total	50	2	8	10	20%

Gall stone culture was positive in 10 cases out of 50 (20%),of which maximum were in age group of 51-60 ,4/11(36.3%).[Table-11]

TABLE 12 -Incidence of Gallstone culture positivity in male and females

SEX	Gallstone CULTURE POSITIVITY
MALE	2/13 (15.3%)
FEMALE	8/37(21.6%)

Gallstone culture was positive in 2 cases out of 13 and in 8 female patients out of 37.Male comprising of 15.3% and female comprising of 21.6%. [Table-12]

TABLE 13:-INCIDENCE OF GALLSTONE CULTURE POSITIVITY IN DIFFERENT TYPE OF GALLSTONE

Type of gallstone	Total no.of cases	Positive bile culture	Percentage
Cholesterol	21	1	4.7%
Pigment	27	7	25.9%
Mixed	2	2	100%

Gallstone culture was positive in 7 cases out of 27 case of pigment stone (25.9%),1cases out of 21 case of cholesterol stone (9.5%),and in 2 case of mixed type of gallstone out of 2. [Table-13]

TABLE 14:- COMMON ORGANISM ISOLATED FROM GALLSTONE CULTURE

ORGANISM ISOLATED	Gallstone culture positivity	Percentage
E.Coli	9	90%
Klebsiella	1	10%
Pseudomonas	0	0
Bacteriods	0	0
Staphylococci	0	0
Streptococci	0	0
Salmonella typhi	0	0

The most common organism isolated from gallstone was E.coli. [Table-14]

TABLE 15-ASSOCIATION BETWEEN BILE CULTURE AND GALLSTONE CULTURE.

Only Bile culture positive	Only Gallstone Culture Positive	Gallstone +Bile Culture Positive
7	2	8

In present study in 8 cases both bile culture and gallstone culture was positive. [Table-15]

Table 16:-Histopathology

Histopathology	No. of cases
Chronic cholecystitis	50
Metaplasia	0
Dysplasia	0

The histopathology report was suggestive of chronic cholecystitis in all the cases. [Table-16]

DISCUSSION

In **present** study pigment type of stone was predominant, which was found in 27 (54%) cases out of 50. Next common gallstone found was cholesterol type which was found in 21 (42%) cases out of 50. Mixed type gallstone was only found in 2 cases out of 50. Shaffer 2006 The most common type of stone in developed countries are composed primarily of cholesterol.⁶ However literature from **Chennai-Jayanthi v et al 1998**,⁷ majority of gallstone found was pigment type (65%).

The variation in different types of gallstones varies significantly in different parts of India. Food habits may be one of the main reasons. Cholesterol gallstones are predominant in the northern, eastern and western parts of India, while pigment gallstones are common in the southern region **M. Ashok et al 2003** ⁸, **Jayanthi et al.**⁷

In majority of studies it is found that bile in normal patient is sterile. **Attila Csends and Patriaco Burdiles** ⁹ from Department of surgery, Santiago Chile found that no patient had bacteria in bile in control case. Among the 165 with symptomatic gallstone disease 52 (32%) had pathogens in their bile, and among the 46 with acute cholecystitis the corresponding figure was 19 (41%) while among patients with common bile duct stones this figure was 39 (58%). Among the 58 patients with carcinoma of the gallbladder the bile grew organism in 47 (81%). Patients over the age of 60 years tended to be more likely to have organism in their bile than patients aged 60 or less, and the difference was significant for symptomatic gallstone disease. Common bacteria isolated was E.coli 52%. Result of present study was similar to **Ondan H et al**¹⁰ study in which maximum bile culture positivity were in pigment stone (65%).

Type of infection In present study Monomicrobial infection was present in 93.3% of cases and Polymicrobial infection was present in 6.6%, which was similar to **Mohammad Moazenia et al** ¹¹ in which monomicrobial infection was 96%. **Most common Isolated Microorganism**- In present study **E.coli** was the most common organism, isolated in 11 sample (66.3%), followed by Klebsiella 3/15 (20%). Anaerobic organism was not found. In various other studies also E.Coli was the commonest organism.

In a study by **Ballal et al 2001**.¹² In India- of the 25 gallstone processed, 6 yielded aerobic bacteria (25%) which were similar to the isolates in bile culture from the same patient. The organism most common isolated was E.coli.

In present study, Incidence of culture positivity was maximum in cases of pigment type of gallstone, 7 out of 27 (25.9%) and in mixed stone both were positive (100%) and in cholesterol stone culture was positive in 1 out of 21 cases (4.7%). Incidence of positive cases were more in mixed type of gallstone, but this could not be supported due to less case mixed type of gallstone.

This result was approximately similar to that of Howard and Kauffman¹³, Cetta et al ¹⁴ and Stewart et al ¹⁵ study. In a study by Ballal et al 2001.¹² in India- Of the 25 gallstone processed, 6 yielded aerobic bacteria (25%) which were similar to the isolates in bile culture from the same patient. The organism most common isolated was E.coli.

Cholelithiasis produces diverse histopathological changes in the gallbladder mucosa namely acute inflammation, chronic inflammation, glandular hyperplasia, granulomatous inflammation, cholesterosis, metaplasia, dysplasia and carcinoma.

Study by Piehler and Crichlow ¹⁶ was showed that more than 70% patient developing gallbladder carcinoma have gallstone in their gallbladder. The risk of developing carcinoma is about 1% in calculous gallbladders 20 years after the initial diagnosis of gallstones.

In **present study** all the cases histopathology was suggestive of chronic cholecystitis. No cases show dysplasia or metaplasia. **P.Hazarah et al 2004** ¹⁷-showed organism were cultured from gall stone in 77% of gallbladder carcinoma.

Carcinoma of gallbladder is highly prevalent in South Asia, and it is here that non cholesterol stones (stone due to infection) are also common. Increasing age is common risk factor in gallbladder carcinoma. High rate of positive bile culture and of calcified stone have been found in elderly.

Infection has been minor risk factor for carcinoma of gallbladder but data are sparse. It is possible that chronic colonization of the gallbladder with gallstone harbouring enteric bacteria may predispose to gallbladder carcinoma in elderly, but further studies are required before definitive conclusion can be drawn in this regard.

Conclusion

The present study was conducted to study the role of bacteria in lithogenesis of gallstone, for which both bile and gallstone were subjected for culture, as only bile culture could not really suggest the presence of bacteria in gallstone which may have initiated lithogenesis, due to two fallacies :-

Firstly the culture of organism from the bile at time of surgery was no necessary indicative of cause effect relation between infective microorganism and lithogenesis, as infection may be secondary to calculus.

Secondly the failure to isolate organism from bile also does not indicate etiology in lithogenesis unrelated to infection as it is well known that organism which initiate stone precipitation may not present in viable form till surgery.

So for this culture of organism from gallstone need to be necessary and correlating of it with culture of microorganism of that of bile.

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