

MICROFLORA OF GALL BLADDER CONTENTS IN PATIENTS UNDERGOING LAPAROSCOPIC CHOLECYSTECTOMY IN ACUTE AND CHRONIC CHOLECYSTITIS.

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ABSTRACT:

Background: According to the statical data maximum number of the surgical operated patients are cholecystectomy patients with gall stone having the major cause of the disease. Acute and chronic inflammation of the gall bladder is the most common complication of the gallbladder stone disease. A bacterial cause has been proposed and bacteria are cultured in most of the patients with acute cholecystitis. While the reported incidence of positive bile culture in patient with chronic cholecystitis who had undergone elective operation on the other hand is less, whereas evidence also suggests that normal bile is sterile. So identifying the microflora will help to identify the probable cause and to choose appropriate antibiotics.

Methods: A prospective study was done on 80 patients for a period of one year of surgery in association with microbiology department, all the patients undergoing laproscopic cholecystectomy acute and chronic were taken for taken, bile gall bladder wall and the stones were sent to the laboratory for culture.

Results: In the present study 80 patients were evaluated. Out of which maximum number of patients were clustered between 35-45 years (25 patients), 70(%) were females and 10 (%) were

males. We found 31.25% bile culture positive, 51.25% gall stones were positive and gall bladder wall was positive for culture in 57.5% The commonest organism was Staph Aureus(35%) then E. coli (10%) followed by streptococcus (7.5%)

Conclusions: Person with the biliary tract disease harbor bacteria in the gall bladder stone , bile and the wall of the gall bladder , so it's important in choosing the antibiotic to prevent complication in only minimum number of the patients and as the stones also harbor the microorganism so the stones should not be handed over the attendants' and the patients.

Keywords: Acute Cholecystectomy , Bile cultures , Gall bladder stones.

INTRODUCTION:

Acute and chronic inflammation of the gall bladder is the most common complication of the gallbladder stone disease. A bacterial cause has been proposed and bacteria are cultured in most of the patients with acute cholecystitis.¹ While the reported incidence of positive bile culture in patient with chronic cholecystitis who undergo elective operation on the other hand is less². Where as evidence suggest that in a normal gallbladder, bile is sterile (Cuschieri et al. 1988). Aspiration and culture of bile at the time of surgery has provided a unique opportunity to study the bacterial flora, as this may also have diagnostic, prognostic or therapeutic implications. The biliary infection can be caused by any type of organism ranging from aerobic gram positive to aerobic gram negative to anaerobic organisms³. The micro organisms present in infected bile are predominantly gram negative aerobes like E.coli, Klebsiella, Proteus, and Pseudomonas. The gram positive cocci are also present in substantial numbers like Staphylococci, Streptococci and Enterococci⁴. Inflamed gall bladder has marked altered permeability which permits absorption of bile acids and movement of inorganic salts into gall bladder lumen. The excessive cellular debris and increased protein secretion, effects consistency of bile which alters its solubility, leading to precipitation of bile salts. By composition Gall stones are of 2 main types, pure stones (Cholesterol, Pigment & Calcium carbonate stones) and Composite stones (Mixed & Combined), most of the gall stones are composite in nature and harbor bacteria in them. The precise origin of the bacteria is not known. Scott (1967) laid down the possible explanation including, ascending infection from duodenum, lymphatic spread and vascular spread or seeding from chronically inflamed gall bladder. The bacteria were also found in the pigment stones. When cultured, bacteria were present in about in 80.5% of those studied. Enterococci were the most common organism that was isolated. Scanning with the electron microscopy showed the presence of the bacteria in the 84.2% of the stones. The bacteria were seen embedded within an amorphous matrix in alternating layers of flake like crystals. (Leung, et al. 1989) Although the exact contribution of bacteria in lithogenesis is not known. It is important for the clinician to realize that most gall stones are likely to be colonized of bacterial biofilm, even though the bile may be culture negative. The chronic inflamed gallbladder wall also contain bacteria. So it was concluded that, in the gallbladder stone disease, bacteria are not only present in bile, but also present in gall bladder wall and gallbladder. As the bacteria are mostly present in gall bladder wall, so stone, thickened gallbladder should be removed even in asymptomatic or symptomatic cases in order to prevent cholecystitis⁶.

METHODS:

It was a prospective carried out in the Medical College for one year. 80 Patients admitted to the hospital, 30 in the emergency as acute cholecystitis with gall stones and 50 through the outpatient department basis who are scheduled to undergo laparoscopic cholecystectomy for chronic cholecystitis. The objective of the study was to determine the frequency of infection in cholelithiasis and to find out the common infecting organism by culturing gallbladder stone, gallbladder wall and gallbladder bile and correlating them in genesis of stone disease and to know the various types of stones present in the patients of gall stones with associated symptoms and post operative complications after laparoscopic cholecystectomy. To suggest remedial measure depending upon culture in order to avoid complications and associated morbidity in patients of cholelithiasis and acute cholecystitis

INCLUSION CRITERIA:

Acute Cholecystitis within first 5 days of admission to hospital. Chronic Cholecystitis admitted to hospital for elective cholecystectomy not receiving any antibiotics 7 days prior to surgery

EXCLUSION CRITERIA:

Patient with choledocolithiasis, dilated CBD, abnormal liver function tests and renal function tests with and without jaundice was excluded. Those patients in which conversion to other procedure will be done were excluded. All the patients were subjected to laparoscopic cholecystectomy as per uniform standard procedure.

STUDY VARIABLES:

Number and location of port site. Disposable and non disposable trocars. Port site used for removal of gall bladder. Methods of skin preparations and closure.

COLLECTION OF SPECIMEN:

After the completion of cholecystectomy when the gallbladder neck is brought out through abdominal port a sample of bile 2-4 ml was aspirated from the gall bladder using a sterile syringe and sent for culture and sensitivity. The gall bladder was then opened, washed with normal saline after taking out the stones a portion of washed mucosa was transported in nutrient broth and sent for culture and sensitivity in sterile plastic jars. The stones taken out from gall bladder were rinsed with sterile saline solution to remove surface contaminants and blot dried. The stones were then crushed and transported in nutrient broth for culture and sensitivity to the Microbiology lab without delay for processing and testing.

CULTURE AND IDENTIFICATION OF ORGANISMS:

All the samples were plated onto duplicate sets of MacKonkey agar and Blood agar plates using a uniform standard procedure which were then incubated at 37degree C in a microphillic atmosphere using a candle jar over night. The bacteria isolated from the cultures were identified with standard microbiological methods by gram staining and biotyping. The Sensitivity was tested on Muller Hinton by Kirby-Bauer Method. Patients were discharged from hospital on 2nd and 3rd post operative days. In some cases hospital stay was prolonged due to some complications. All the culture reports were collected and analyzed to determine the causative

organism, the type of the stone. Patients were followed up in the postoperative period for: Pain, wound, infection, Intra-abdominal abscess, fever and septicemia.

RESULTS:

In the present study 80 patients were evaluated for culture and gall bladder bile with the men age 37.95 years (16-64)

Table 1: Number of patients according to age distribution.

Age	Number	Percentage
15-24	8	10
25-34	22	27.5
35-44	25	31.25
45-54	17	13.6
55-64	8	10

Present study showed highest percentage of people in age group 35-44. In the present study out of 80 patients, 70 (87.50%) were females and 10 (12.50%) were males.

Table 2: Signs and symptoms of the patients

Clinical Presentation	Acute Cholecystitis N=30	Chronic Cholecystitis N=50	Total N=80
Abdominal Lump	30[100%]	46[92%]	76[95%]
Dyspepsia	24[80%]	21[42%]	45[56.3%]
Vomiting	20[66.7%]	0[0%]	20[25%]
Abdominal Lump	7[23.4%]	3[6%]	10[12.5%]
Jaundice	0[0%]	1[2%]	1[1.3%]

Table 3: Percentage of the growth of organisms in bile. The organisms were positive in 31.25% of the cultures.

ORGANISM	Acute cases N=12/25	Chronic cases n=13/50	Total N=25/80
Staph.aureus	4(33.4%)	5(38%)	9(36%)
E.coli	3 (25%)	4(30.7%)	7 (28%)
Klebsiella	2 (16.8%)	--	2 (8%)
Salmonella	1 (8.4%)	1 (7.6%)	2 (8%)
Pseudomonas	--	1 (7.6%)	1 (4%)
Micrococcus	--	1 (7.6%)	1 (4%)
Citrobacter	--	1 (7.6%)	1 (4%)
Streptococcus	1 (8.4%)	--	1 (4%)

Acinetobacter	1 (8.4%)	--	1 (4%)
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Table4: Percentage of the growth of organisms in Gall bladder wall. 46.66% cases of acute cholecystitis were positive and 50% cases of chronic cholecystitis were positive for wall culture.

ORGANISM	Acute cases	Chronic cases	Total
	N=14/30	N=25/50	N=39/80
Staph. aureus	5(29.4%)	9(40%)	14(35%)
Aerobic spore bacillus	2(17.7%)	4(18%)	6 (15%)
E.coli	3 (12%)	1(4.5%)	4 (10%)
Streptococcus	1(6%)	2(9%)	3(7.5%)
Pseudomonas	1(6%)	2(9%)	3(7.5%)
Micrococcus	1(6%)	2(9%)	3(7.5%)
Salmonella	1(6%)	1(4.5%)	2 (5%)
Acinetobacter	--	2(9%)	2 (5%)
Yeast	--	1(4.5%)	1(2.5%)
Klebsiella	--	1(4.5%)	1(2.5%)

Table5: Percentage of the growth of organisms in Gall bladder stone. Out of 80 patients there were 41 cases (51.25%) which were having, stone culture positive. In acute cases 56.7% cases were positive for culture where as 48% were positive in chronic cases

Organisms	Acute	Chronic	Total
Staph. aureus	8(40%)	6(33.4%)	14(34%)
ASB	1(6.6%)	6(25%)	7(17.1%)
E.coli	2(13.2%)	2(8.4%)	4(9.7%)
Streptococcus	1(6.6%)	2(8.4%)	3(7.2%)
Citrobacter	--	1(4.2%)	1(2.4%)
Salmonella	1(6.6%)	1(4.2%)	2(4.8%)
Pseudomonas	1(6.6%)	1(4.2%)	2(4.8%)
Acinetobacter	2(13.2%)	--	2(4.8%)
Micrococcus	--	1(4.2%)	1(2.4%)
Yeast	--	1(4.2%)	1(2.4%)
Klebsiella	2(13.2%)	--	2(4.8%)
Diphtheroids	1(6.6%)	--	1(2.4%)
Staph. Albus	--	1(4.2%)	1(2.4%)

Table 6: Percentage of the stones removed from the gall bladder, combined and mixed stones constitute the maximum numbers with 63.78% stones

Types of stones	No of stones N=80
Mixed	44(44.54%)
Combined	19(19.24%)
Pigment	9(9.10%)
Cholesterol	6(6.8%)
Calcium carbonate	2(2.3%)

Figure 1-4 shows Cultures of E Coli, Staphylococcus aureus, Micrococci and Acinetobacter.

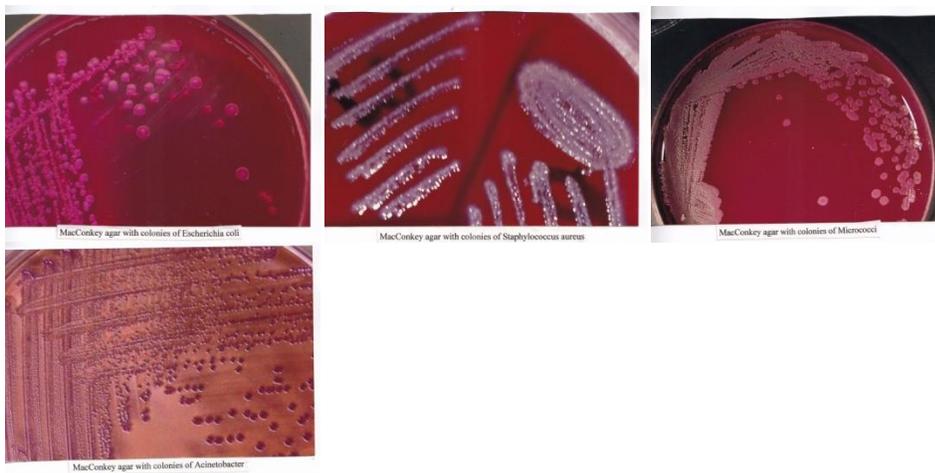


Figure 5 and 6 shows various types of gall stones .



Various types of gall stones



Mixed gall stones

DISCUSSION:

80 patients undergoing laparoscopic cholecystectomy were studied. Mean age of the patients were 37.95 yrs (range 15-64 yrs). Consistent with the findings of previous studies^{7,8,9}. In our series we observed that females outnumbered the males. We had 70 females undergoing laparoscopic cholecystectomy as against only 10 males, with female to male ratio of 7:1. In the literature similar findings were reported Graves et al (1991)

Edlund (1959)¹⁰ studied 300 cases of acute and chronic cholecystitis and found 70% to be culture positive in acute cholecystitis and in chronic cholecystitis it was less than 50%. Stone et al (1977)¹¹ isolated organism from bile in 71.44% of acute cholecystitis cases and 54.6% of chronic cholecystitis cases. Brismar et al (1986)¹² in his study on 84 patients observed bile culture positivity in 62.5% cases of acute cholecystitis while only 47% cases were culture positive for chronic cholecystitis. As the gallbladder wall is always involved in the process of both acute and chronic cholecystitis, so bacteria are presumed to be present in the wall of the gall bladder in addition to the gallbladder bile. In our series the gall bladder wall has shown 50% and 42% positive culture for acute and chronic cases respectively. Our study is consistent with other studies like Haneke (1986)⁶ who in their study observed 57.1% gallbladder wall culture in acute cases and 11.5% in normal patients. In our study the gall bladder stone culture showed 56.7% and 48% positive culture for acute and chronic cases respectively wherever Beugen (1979) found 28%, Hazrah (2004)⁹ found 81% positive and Haneke (1986)⁶ observed 20% gall bladder stone

cultures positive. In our series Staphylococcus cases followed by E.coli in 25% Klebsiella in 16.8%, Salmonella in 8.4%, Streptococcus in 8.4% and Acinetobacter (8.4%) cases The organism recovered from bile in chronic cases were Staphylococcus aureus in 38% cases followed by E.coli ,30.7%, Salmonella in 7.6%, Pseudomonas in 7.6%, Micrococcus in 7.6% and Citrobacter in 7.6% cases.

The compiled data revealed that gram negative organism predominate in our study as compared to gram positive organism which primarily included Staphylococcus aureus and Streptococcus. Ludwij J (1964)¹³ , Van Leeuwen (1985)¹⁴ Brook. (1989)¹⁵ , Sakurai et al. (1992)¹⁶ and I Sattar, A Aziz et al (2007)¹⁷ in their studies mentioned E.coli to be predominant organism. One study by Edlund (1959) conducted on 300 operated cases, which revealed gram positive organism Streptococcus culture positivity in acute cholecystitis to be 75% and in chronic cholecystitis it was less than 50%. Where as E.coli was next organism cultured. In our study culture positivity form gall bladder wall was to the tune of 57.5% which was more than culture positivity of bile 31.25% and gall bladder stone 51.25% similar pattern was obtained proving the fact that gall bladder wall harbored larger number of bacteria than bile, the high incidence of gall bladder wall culture is a significant evidence to the fact that gallbladder should be removed in order to prevent relapsing cholecystitis as stated similarly by (Hanke 1986)⁶ and Sakurai et al (1992)¹⁶

Staphylococcus aureus in isolation was found in 35% specimen the others were Aerobic SporeBearer (15%), E.coli (10%), Streptococcus (7.5%), Pseudomonas(7.5%), Micrococcus (7.5%), Salmonella (5%), Acinetobacter (5%),Yeast(2.5%) and Klebsiella (2.5%) The organisms recovered in acute cases in our study were mostly of the enteric origin. The gallbladder stone showed a total of 51.25%.

Salmonella and yeast are a present in the stones in an active state, 2 cases 4.8% in our study were positive for Salmonella. So it suggests that stones should not be handed over to the patient for the fear of transmitting infection.

80 patients, 48(60%) patients had multiple stones, 26(32.5%) had single stones and 6(7.5%) patients had 2 stones obtained from the gall bladder similar to the findings of Chunhamaneewat findings (1999)¹⁸.

Similar results were as of our series were shown by Tarek S Malatani T(1996)¹⁹ who in their study of 41 patients found 21 stones (51%) were of mixed type, 15 stones (57%) pigmented and 5 stones (12%) cholesterol. Whereas Vasitha et al (2008)²⁰ observed 35 cholesterol stones and 35 pigment stones from 51 patients and isolated bacteria from pigment stones of 29(82%) and from cholesterol stones of 9 (26%).

25 of our patients presented with postoperative pain for 1-2 days which was due to the port site incision and pneumoperitonium. They were alright by the end of 2 days with the use of analgesic (diclofenac sodium) 3 patients developed low grade fever but they improved without use of any antipyretics. 4 patients (5%) developed port site infection and all of them were minor infections. The distribution of organisms in them was Pseudomonas in two, staphylococcus aureus and Klebsiella in the others. Similar findings were reported by Den Hoed et al (1998)²¹

This series also confirmed the work of others that the presence of bacteria in the bile did not seem to influence the incidence of wound infection. There was no sepsis, Intra-abdominal

abscess and death indicating that laparoscopic cholecystectomy is a very safe procedure in expert hands in spite of gallbladder harboring many organisms.

CONCLUSION:

1. Females are affected far more than males.
2. The mean age of presentation is around 25-35 years and most of them get operated at 30-40 years of age.
3. Pain was the most common symptom in our study both in acute and chronic cholecystitis followed by dyspepsia.
4. Over all gram negative organisms predominated in gall bladder stone, gall bladder wall and gall bladder bile.
5. In Acute cholecystitis culture positivity is much more than chronic cholecystitis cases referring to the infective process of the disease
6. Staphylococcus aureus was the most common isolated bacteria in our study followed by E.coli
7. Culture should not be routinely done in patients undergoing biliary tract surgery as it has got no implication on the mode of treatment and is not cost effective. Stone should never be handed over to the attendants or the patients as it may be the source of infection.

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Conflict of interest: None declared.

Ethical approval: The study was approved by the Institutional Ethics Committee.

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