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Original Research Article

Bacteriological Profile of Bile in Patients Undergoing Cholecystectomy

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Abstract: Cholecystitis and cholelithiasis are frequently met surgical conditions in North India. The presence of bacteria in bile at the time of surgery or invasive diagnostic procedures predisposes to complications like septicaemia. Aim of this study was to study the bacteriological profile – aerobic & anaerobic in the bile of patients undergoing cholecystectomy. The present study included 50 patients admitted in the department of general surgery undergoing cholecystectomy with preoperative diagnosis of biliary tract disease. Details of the operative findings were recorded. The bile from gallbladder was cultured for the presence of aerobic and anaerobic bacteria. The bacteriological examination of the bile yielded positive culture in 16 out of 50 cases (32%). Aerobic bactobilia was seen in 15 out of 16 patients (93.75%). Anaerobic bactobilia was seen in 4 out of 16 patients (25%). The infection was mostly due to single microorganism. In 9 out of 16 cases (56.25%) the growth was purely aerobic. E.Coli was the most commonly isolated aerobic bacteria (36%).
Keywords: Cholelithiasis, Cholecystitis, Bactobilia, Escherichia Coli, Aerobic Bacteria, Anaerobic Bacteria

INTRODUCTION

Cholecystitis and cholelithiasis are frequently met surgical conditions in North India[1] and is one of the most common diseases affecting the gastrointestinal tract.

The presence of bacteria in bile at the time of surgery or invasive diagnostic procedures predisposes to septic complications like septicaemia, liver abscess, hepatic and renal failure, endotoxaemia and disseminated intravascular coagulation and hence the judicious use of prophylactic antibiotics reduce morbidity and mortality due to infection.

The various concepts of pathogenesis of infection of biliary tract are based on four possible routes.

1. Haematogenous infection
2. Descending infection from the liver (Enterohepatic route).
3. Ascending infection from duodenum upto the CBD.
4. Spreading infection via lymphatics through the wall of gallbladder.

Haematogenous Infection

Haematogenous spread of infection to gallbladder occurs either via arterial route as bacterial emboli or general sepsis or through venous circulation as in thrombophlebitis or portal vein thrombosis.

Edmund *et al* [2] reported that there was some evidence that bile could be contaminated by means of portal venous bacteraemia or by translymphatic spread. The type of the bacteria isolated from the biliary tract tend to negate this possibility that organism arrives in gallbladder from general circulation during septicaemia.

Tynes and Utz [3] noticed that cholangitis and cholecystitis occurred after typhoid and cholera as a consequence of transient arterial and portal venous bacteraemia which took place during the disease.

Descending Infection From The Liver (Enterohepatic Route)

Twiss *et al* [4] stated that these micro-organisms might reach the liver through systemic circulation or more readily by way of portal vein and later in the bile, bile ducts and gallbladder. They

reported that culture of liver might be positive for pathogenic micro-organisms in normal persons because some bacteria transmitted by bloodstream to the liver for destruction, might survive. They are excreted into bile ducts. However, they rarely produced pathological changes or symptoms there, except in the presence of obstruction.

Ascending Infection

Ascending infection from intestine via papilla of vater up through the bile ducts to the gallbladder has been studied from time to time.

Edmund *et al* [2] suggested that infection ascends up from the duodenum, supported by the fact that number of bacteria in gallbladder bile was higher than the gallbladder itself.

Spreading Infection Via Lymphatics

Spreading infection via lymphatics takes place through wall of gallbladder from inflamed contiguous organs like colon, liver, stomach and duodenum.

Graham and Peterman [5] established the fact that there was close relation and association between the lymphatics of gallbladder and those which drained the bowel. Moreover, it had long been known that liver receives some of portal lymph drainage through this network.

Organisms responsible for biliary tract infection are bacteria E.Coli, Streptococcus faecalis, Klebsiella, Pseudomonas aeruginosa, fungi and ova of parasites- ascaris lumbricoides and clonorchis sinensis.

The selection of antibiotics for the treatment of active infection and for the prophylaxis of post-surgical sepsis is dependent upon the nature and sensitivity of the pathogens involved. In the vast majority of cases, infection is due to bacteria originating from the biliary tract. The incidence and type of bacteria involved and their susceptibility to antibiotics can be accurately predicted by bacteriological examination of the bile and a suitable antibiotic regimen can then be selected to minimise post-surgical infections.

Most common infection is due to E. coli and typhoid bacilli. These organisms reach the liver by way of portal vein and are then transported via the bile to bile ducts and gallbladder [4].

Various studies have been done to study the bacterial flora of bile and biliary tract but on no other subject such diversity of opinion exists as on the nature of the gallbladder infection. The predominant bacterium according to AlHarbi *et al* [6] is E.Coli while Wayne and Whelan [7] reported Klebsiella as predominant organism in the biliary tract. Other organisms found in the bile include:

Aerobic

Gram positive

Streptococcus faecalis
Streptococcus viridians
Staphylococcus aureus
Staphylococcus epidermidis

Gram Negative

Enterobacter species
Proteus species
Pseudomonas aeruginosa

Anaerobic

Gram Positive

Clostridium perfringens
Anaerobic cocci

Gram Negative

Bacteroides fragilis

Hence the various studies done on bacterial flora of biliary tract present a confusing picture, the present study has been aimed at evaluating the bacteriological profile, both aerobic and anaerobic of patients undergoing cholecystectomy and to proceed accordingly.

AIMS & OBJECTIVES

To study the bacteriological profile – aerobic & anaerobic in the bile of patients undergoing cholecystectomy

MATERIAL & METHODS

The present study included 50 patients admitted in the department of general surgery undergoing cholecystectomy with preoperative diagnosis of biliary tract disease. Details of the operative findings were recorded. Bacteriological study of bile was carried out.

During operation, note was made of state of gallbladder, cystic duct, CBD and their adhesions with surrounding structures. A note was also made of any internal or external CBD obstruction. Details of operative procedure were recorded.

Bacteriological Study:

On operation table, 2ml bile from gallbladder was aspirated with a sterile syringe in the beginning of the exploration for bacteriological studies. Within one hour of collection, bile was sent to the department of microbiology for processing.

Each specimen was inoculated on two blood agar plates and one MacConkey agar plate. One blood agar and MacConkey agar was incubated aerobically at 37^0 C for 24 hours and other blood agar plate with haemin and mennadione incubated anaerobically at 37^0 C for 48 hours in anaerobic jar to see the aerobic and anaerobic growth respectively. All the samples were subjected to smear examination and culture for both aerobic and anaerobic organisms.

Smears were prepared from both specimens and after staining with Gram's Method, were examined for presence of gram negative and gram positive bacteria.

Culture plates were examined after overnight incubation for presence of growth.

Growth was confirmed from colony characters, by Gram's staining, biochemical reactions and any special tests required confirming the particular bacterium.

Aerobic Bacteria

Gram Positive

a) Staph epidermidis: Colony characters- on blood agar, colonies are small, circular, smooth, entire margins, butyrous consistency, white in colour and non- haemolytic. Growth is confirmed by no haemolysis on blood agar, white colour colonies and coagulase test negative.
b) Staph aureus: Colony characters- on blood agar, colonies are smooth, golden yellow in colour, low convex, glistening, densely opaque, butyrous consistency and surrounded by a narrow zone of haemolysis. Growth is confirmed by haemolysis on blood agar, golden yellow colour and coagulase test positive.
c) Strep faecalis: Colony characters- on MacConkey agar, colonies are small (0.5 to 1mm), usually magenta colour and non-haemolytic. Growth is confirmed by mannitol test positive and heat test positive.

Gram Negative

a) E.Coli: Colony characters- on MacConkey agar, colonies are large (2-3mm), circular, low convex, red or pink in colour and opaque. Growth is confirmed by biochemical reactions (indole test positive, methyl red test positive)
b) Klebsiella pneumonia: Colony characters- on MacConkey agar, colonies are large mucoid and red. Growth is confirmed by biochemical reactions (string test positive).
c) Pseudomonas aeruginosa: Colony characters- on blood agar, colonies are large (2-3mm), smooth, translucent, irregularly round with a characteristic fruity odour. Growth is confirmed by production of bluish green pigment (pyocyanin) and oxidase test positive.
d) Proteus: Colony characters- the culture emits characteristic putrefactive (fishy) odour and possess the ability to swarm (spread on solid media). Growth is confirmed by phenylalanine deaminase test positive and urease test positive.
e) Salmonella typhii: Colony characters- on MacConkey agar, colonies are pale yellow or colourless due to absence of lactose fermentation. Growth is confirmed by biochemical reactions. It gives a positive reaction for H_2S in triple sugar iron agar.

Anaerobic Bacteria

Gram Positive

Anaerobic cocci- Peptococcus: Colony characters- on blood agar, colonies are black in colour on prolonged incubation. Growth is confirmed by biochemical reactions (Catalase test negative and indole test negative).

Gram Negative

Bacteroides fragilis- Colony characters- on blood agar, colonies are 1-2mm, smooth, low convex, translucent or light grey with no haemolysis. Growth is confirmed by biochemical reactions (indole test positive).

RESULTS AND OBSERVATIONS

The present study was conducted on 50 patients who had undergone cholecystectomy for biliary tract disease. The patients suffering from malignant diseases of the biliary tract were excluded from the present study.

Operative Findings

The operative findings showed the gallbladder to be contracted in 72%, thick walled in 82% of cases and there were thick adhesions around it in 36% of cases. The stone in cystic duct was observed in 2% cases and in Hartman's pouch in 6% cases. The CBD was dilated in 14% cases but stone could be seen in 8% of cases.

Incidence Of Infection In Bile From Gallbladder

Out of 50 patients, 16 patients had positive bile culture thus accounting for an incidence of 32%.

Incidence Of Gallbladder Bile Culture Positive Cases

Out of 50 cases, infection was seen in 16 cases only. Positive bacterial cultures were obtained from bile of gallbladder. The overall incidence of aerobic infection in bile in the present study was 93.75% (15/ 16 cases) and anaerobic infection in bile was 25% (4/ 16 cases).

In our study, patients were in the age range of 17-65 years, the incidence of bactobilia was found to be related to the age of the patients. Incidence of positive bile culture in 3^{rd} decade was 14.2%, in 4^{th} decade was 25%, in 5^{th} decade was 41.6%, in 6^{th} decade was 50% and in 7^{th} decade was 63.3%. Incidence of bactobilia was found to be increasing with age.

A total of 8 types of aerobic bacteria and 2 types of anaerobic bacteria were isolated from 16 patients as shown in the above table. Among the aerobic bacteria E.Coli was predominantly grown. Anaerobic isolates were anaerobic cocci and bacteroides fragilis.

Table-1: Relationship between Age of Patients and Bactobilia

Age Group	Total No. Of Cases	Bactobilia	%Age
11-20	2	0	0%
21-30	7	1	14.2%
31-40	16	4	25.0%
41-50	12	4	41.6%
51-60	10	5	50.0%
>61	3	2	63.3%

Table-2: Biliary Bacteriological Spectrum

Type of Growth	Type of Organism	No. of Cases	%Age
AEROBIC	*E. Coli*	9	36%
	Staph. Epidermidis	3	12%
	Klebsiella Pneumonia	2	8%
	Pseudomonas Aeruginosa	2	8%
	Streptococcus Faecalis	2	8%
	Proteus Mirabilis	1	4%
	Staphylococcus Aureus	1	4%
	Salmonella	1	4%
ANAEROBIC	*Anaerobic cocci*	3	12%
	Bacteroides fragilis	1	4%

Bacterial Flora

Out of 16 patients, who had bactobilia, 9 had pure aerobic growth (56.25%), 1 had pure anaerobic (6.25%) and 6 mixed growth (37.50%).

Mixed bacterial flora was seen in six cases. Among the mixed flora, 3 had only aerobes and the remaining 3 had both aerobes and anaerobes in them.

Out of 16 patients, in 10 a single bacterial species was isolated from the bile (62.50%). 6 patients had polybacterial growth (37.50%).

Table-3: Mixed Flora Isolated From Bile Samples

Mixed Flora	No. of Cases	% Age
E.Coli+ Salmonella	1	16.66%
E.Coli+ Anaerobic cocci	2	33.33%
Staph epidermidis+ Pseudo. aeruginosa	1	16.66%
Staph. Epidermidis+ Kleb. Pneumoniae+ Anaerobic cocci	1	16.66%
E.Coli+ Pseudo aeruginosa+ Staph epidermidis+ Strep faecalis	1	16.66%

Table-4: Quantum of Growth

No. of species isolated	No. of cases	%age
Single	10	62.50%
Two	4	25.00%
Three	1	6.25%
Four	1	6.25%

DISCUSSION

The present study was carried out on 50 patients of biliary tract disease who were admitted and operated for gallstone disease. The patients suffering from the malignant diseases were excluded from the present study.

Bacteriological Study of Biliary Tract

The subject pertaining to gallbladder infection presents a great diversity of opinion. In 1892, Naunyn emphasized the importance of infection in the causation of gallbladder disease and advanced his theory 'Lithogenic Cattarh' of the gallbladder. Since then the contribution of infection in the causation of gallbladder disease has been admitted.

Incidence of Infection in Bile

In the present study, aerobic bacteria were grown in 15 cases (30%) from the gallbladder bile. The thirty percent positive aerobic culture incidence in the present study is quite comparable to that reported by Chang *et al* [8] - 36%.

In the present study, anaerobic bacteria were grown in 4 cases (8%) from the gallbladder bile. The 8% positive anaerobic culture incidence is quite comparable to that reported by Claesson *et al* [9] -11%.

Bile Infection in Jaundiced Patients

In the present study, the incidence of infection in cases with jaundice (with or without stone in CBD) who were admitted to surgery was 66.67%. Higher incidence has been reported by Flemma *et al* [10] – 90%.

Bacterial Spectrum

In all the cases, inoculations for aerobic and anaerobic bacteria were carried out. The growth of aerobic microorganisms was obtained in 30% cases and anaerobic microorganisms were obtained in 8%.

Bacterial profile of the organisms isolated Aerobic

E.Coli was the most common organism isolated -36%.

Other aerobic bacteria isolated in order of frequency were *Staph. Epidermidis, Klebsiella Pneumonia, Pseudomonas Aeruginosa, Streptococcus Faecalis, Proteus Mirabilis, Staphylococcus Aureus, Salmonella.*

AlHarbi *et al* [6], has also reported *E.Coli* as the most frequent organism isolated from the bile -28.1%.

Staph epidermidis was isolated from 12% cases of bile culture. This figure is quite comparable with the figures projected by AlHarbi *et al* [6] -12.5%.

Klebsiella pneumonia was cultured from 8% cases. This figure is quite comparable with the figures projected by AlHarbi *et al* [6] -6.3%.

Pseudomonas aeruginosa was cultured in 8% cases. This figure is quite comparable with the figures projected by AlHarbi *et al* [6] -9.4%.

Streptococcus faecalis was isolated from 8% cases. This figure is quite comparable with the figures projected by Claesson *et al* [9] - 9.3%.

Proteus mirabilis was isolated from 4% cases. This figure is quite comparable with the figures projected by Csendes *et al* [11] – 6%.

'Salmonella was isolated from 4% cases. This figure is quite comparable with the figures projected by Csendes *et al* [11] – 4%.

Staph aureus was isolated from 4% cases. This figure is comparable to the results reported by Lou *et al* [12] - 5%.

Anaerobic

Anaerobic cocci was the most common anaerobic organism isolated, *Bacteroides Fragilis* has been isolated from one case in our study. None of our patients had biliary tract colonised by *Clostridia*.

Anaerobic cocci had also been isolated by Treudson [13]. Fu [14], reported *Bacteroides Fragilis* as the frequent anaerobic isolate.

SUMMARY & CONCLUSIONS

50 patients admitted in general surgery department for gallbladder disease were subjected to surgery. The bile from gallbladder was cultured for the presence of aerobic and anaerobic bacteria and the following conclusions were made:

1. The bacteriological examination of the bile yielded positive culture in 16 out of 50 cases (32%).
2. Aerobic bactobilia was seen in 15 out of 16 patients (93.75%).
3. Anaerobic bactobilia was seen in 4 out of 16 patients (25%).
4. The infection was mostly due to single microorganism.
5. In 9 out of 16 cases (56.25%) the growth was purely aerobic while in 1 out of 16 cases (6.25%) the growth was purely anaerobic.
6. Mixed bacterial flora was found in 6 out of 16 cases (37.50%). Among the mixed flora, 3 had only aerobes and the remaining 3 had both aerobes and anaerobes.
7. In 10 patients a single bacterial species was isolated from the bile (62.50%). 6 patients had polybacterial growth (37.30%)
8. A total of 8 types of aerobic bacteria were isolated from 16 patients. E.Coli was the most commonly isolated aerobic bacteria (36%) followed by Staph Epidermidis (12%) and closely followed Strep Faecalis, Klebsiella Pneumonia and Pseudomonas Aeruginosa in equal incidence (8%). The less common infective aerobes were Proteus Mirabilis, Staph Aureus and Salmonella in equal incidence (4%).
9. A total of 2 types of anaerobic bacteria were isolated from 16 patients. Anaerobic cocci was the most commonly isolated anaerobic bacteria (12%) followed by bacteroides fragilis (4%).

Thus, to conclude, biliary cultures were positive in significant number of cases (32%) with enterobacteria being the most frequent isolate. Though our series is probably too small to draw emphatic conclusions, still it seems that infection plays an important role in biliary tract disease.

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