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A Bacteriological Study of Bile in Gall Bladder Disease : A One Year Study in a Tertiary Care Centre in Assam, India

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ABSTRACT

Cholecystisis and cholelithiasis with its complication dominate the disease of the biliary tract.These diseases may cause severe infection and/or sepsis. In addition to surgical treatments, prompt administration of appropriate antibiotic is important to control the biliary tract infection. The purpose of this study is to illustrate the bacteriology in biliary tract disease and provide information for antibiotic choices. To isolate the bacterial flora from bile of gallbladder diseases and to determine the antimicrobial susceptibility pattern of different bacterial isolates. In this prospective study of one year study period from July 2006 to June 2007, a total of 50 bile samples was collected from patients undergoing cholecystectomy for gall bladder diseases in Surgical OT and isolated bacteria were identified and their antimicrobial sensitivity pattern studied by standard microbiological methods in a tertiary care hospital in Guwahati, Assam over a period of one year. Out of 50 bile samples, 24 (40%) showed positive bile cultures, the commonest aerobic organisms being E. coli and Klebsiella spp. Bacteroides fragilis was common among anaerobes. Gentamicin was the most effective drug among aerobes. Metronidazole, Chlorumphenicol & Imepenum being effective among anaerobes . Our bacteriological findings shows a changing trend in the microbial etiology and their antibiogram pattern which will help the clinicians to formulate a proper and appropriate antibiotics used against pathogens, to reduce likelihood of complications like wound infections and gram negative septicemia.

Keywords: Cholelithiasis, Cholecystectomy, Antibiogram

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INTRODUCTION

Cholecystisis and cholelithisis with its complication dominate the disease of the biliary tract. Gall bladder disease has a wide geographic variation, being common in the US where an estimated 15 million people have gall stones and of these 1/5 or 3 million undergo biliary tract operations every year. [1] In India, chronic cholecystitis and cholelithiasis are very common diseases of North India.They are seven times more common in the North as compared to the South India.[2] Biliary obstruction is a major risk factor for infectious complication in connection with biliary diseases and following biliary tract surgery. Patients undergoing biliary tract operations are at a higher risk of developing post-operative infection if bacteria are present in their bile at the time of operation.[3] The rate of post-operative wound infection after elective cholecystectomy has been reported to range from 7-20%. [4] Bacterial inflammation plays a role in 50 to 80% of patients in acute cholecystitis and found bacteria in 19.9-46% of patient's bile undergoing cholecystectomy for gall stones. [5] The organisms most frequently isolated by culture of gallbladder bile in these patients include Escherichia coli, Klebsiella spp, Group D streptococcus, Staphylococcus spp and Clostridium spp. [6] The percentage of anaerobic bacteria among positive bile cultures has gradually increased, probably due to improvements in culture techniques. [7] reviewed anaerobic biliary tract infections and observed that anaerobes were recovered from 1 to 16 % of bile cultures and anaerobic bacteria are now identified in some 20-30% of positive cultures, mainly Clostridium and Bacteroides species.[8]Brody et al(1998) investigated clinical factors associated with positive cultures during biliary surgery and concluded that the bile cultures provide valuable information for planning, antibiotic prophylaxis and treatment and that patients with certain clinical high risk factors are more likely to have positive bile cultures and should be given prophylactic antibiotics. [9]

MATERIALS AND METHOD

The samples of bile for bacteriological study were collected from 50 cases during cholecystectomy for acute cholecystitis, chronic cholecystitis and cholelithiasis in surgical OT, GMCH and study was carried out in the Department of Microbiology, Gauhati Medical College, Guwahati, for a period of one year from July 2006 to June 2007. The cases were selected keeping in mind their age, sex, duration of illness, associated complications and pre-operative antibiotics. At surgery, immediately after cholecystectomy, 10-15ml of bile were aspirated with a sterile syringe from the gall bladder; 3-5ml were placed in a sterile universal container, another 5ml were inoculated directly into freshly prepared, pre reduced thioglycollate medium and sent to the Microbiology Department for further processing. The delay in collection and processing was minimal and never

exceeded more than 15 to 20 minutes.Direct examination of specimens was done from the universal containers containing specimens were noted for their amount,odour, colour and presence of granules and deposits.Specimens were centrifuged at 3000 r.p.m for 15 min, smears made from the deposit and heat fixed. Gram staining of the smears was done according to methods described by Duguid (1996) and then cultured aerobically and anaerobically.[10] Media used for isolation of aerobes and facultative anaerobes was 10% Sheep blood agar and Mac Conkey agar and for anaerobic bacteria media used was Blood agar, Gentamycin blood agar, Thioglycollate broth and Robertson cook meat broth (RCM).These media were prepared as per method described by Collee et al, (1996).[11,12] Aanaerobic culture was carried out as per manufacturer's guidelines. (Hi media's anaerobic system.).The anaerobic system used consisted of: Himedia's Anaerobic system Mark 2, Himedia's Anaerobic Pack 3.5L, Himedia's Anaero Indicator tablet.Organisms were identified by standard microbiological methods, which included colony morphology, as well as staining, and biochemical tests.[13,14,15] Pure cultures were tested for antimicrobial susceptibility using the disc diffusion method. Different panels of antimicrobials were used depending on the type of the organism isolated, and results were reported as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. [16]

RESULTS AND DISCUSSION

A total of 50 bile samples was collected from patients undergoing cholecystectomy for gall bladder diseases and investigated during the one year study period.

The study revealed that out of 50 patients of gall bladder diseases, the highest number of cases was in the age group of 31-40 years and the majority of the patients belonged to the female group. The female to male ratio being 3.5:1.Table 1 shows the pathological conditions found in gall bladder diseases where the majority of the cases belonged to the Cholelithiasis group 25(50%) where 24 cases showed multiple calculi compared to only one case of solitary calculus in the gall bladder.

Table 1: Pathological conditions found in gall bladder diseases.

Pathological conditions	Number of patients	Total	Percentage
Cholelithiasis			
Solitary calculi	1	25	50
Multiple calculi	24		
Acute cholecystitis			
Acalculous	2	11	22
Calculous	9		
Chronic cholecystitis	3	14	28
Chronic calculous cholecystitis	11		
Total	50	50	100

The majority of the patients 40(80%) complained of pain abdomen. The associated risk factors were jaundice (30%), >60 years (12%), obesity (10%), diabetes mellitus (12%), choledocholithiasis (14%).

Table 2: Sign / Symptom & Predisposing conditions of gall bladder disease.

S/S &predisposing conditions	Number of patients	Percentage
Pain abdomen	40	80
Nausea &vomiting	30	60
Fever	25	50
Lump abdomen	10	20
Jaundice	15	30
>60 years	6	12
Obesity	5	10
Diabetes mellitus	6	12
Choledocholithiasis	7	14

Out of 50 bile samples, 24 (40%) showed positive bile cultures while 26(52%) were sterile. The total number of isolates were 28, where 20 (71.42%) cultures were aerobes whereas 8 (27.58%) were anaerobes. Table 3 shows that out of the 24 positive cultures, 16 (32%) were strict aerobe, 6(12%) were obligate anaerobes and only 2(4%) cases constituted a mixture of aerobes and anaerobes.

Table 3: Type of culture in positive cases.

Total number of positive cases	Type of Culture	Positive culture	
		Number	Percentage
24	Strict aerobe	16	32
	Obligate anaerobe	6	12
	Aerobe + anaerobe	2	4
Total		24	48

The 24 positive cultures yielded 20(40%) single isolates and 4(8%) multiple isolates in cultures.Of the 20 single isolates, 15 were gram negative bacilli and 5 were gram positive cocci. Of the 4 cultures with mixed bacterial isolates, all were gram negative bacilli.

The predominant organisms among the 20 aerobes were Escherichia coli 5(25%) & Klebsiella spp 5(25%) followed by Coagulase negative staphylococcus at 2 (10%). Single isolate recovered were Staphylococcus aureus, Enterococci, Citrobacter spp, Acinetobacter, Salmonella typhi, Salmonella paratyphi A, Proteus mirabilis and Pseudomonas spp.

Table:4 Number and percentage of aerobic isolates of positive bile cultures.

Aerobic isolates	Isolated in pure forms		Isolated in mixed culture		Total Number	Percentage
	No	%	No	%		
E. coli	4	28.57	1	16.66	5	25
Klebsiella spp.	2	14.28	3	50	5	25
Coagulase negative staph.	2	14.28	-	-	2	10
Staph. aureus	1	7.14	-	-	1	5
Enterococci	1	7.14	-	-	1	5
Citrobacter spp	-	-	1	16.66	1	5
Acinetobacter	1	7.14	-	-	1	5
S. typhi	1	7.14	-	-	1	5
S. paratyphi A	1	7.14	-	-	1	5
P. mirabilis	-	-	1	16.66	1	5
Pseudomonas	1	7.14	-	-	1	5
Total	14	-	6	-	20	100

Out of 8 anaerobes, 7 (87.5%) were Bacteroides fragilis(12.5%) and 1(12.5%) was Peptosteptococcus anaerobius.

Table 5: Number & percentage of anaerobes (GNB/GPC)

Types of isolates	Organisms		No.	Percentage
Anaerobes	GNB	Bacteroides fragilis	7	87.50
	GPC	Peptosteptococcus Anaerobius	1	12.50
Total			8	100

The in vitro antimicrobial sensitivity tests of the bacterial isolates revealed that Gentamicin at 81.25% sensitivity was the most effective antimicrobial agent against the entire spectrum of the gram negative bacilli isolated followed by Ciprofloxacin at 62.5%, Ampicillin at 56.25% and Ofloxacin at 50%.The 5 isolates of E.coli showed the 100% sensitivity to Gentamicin & Ampicillin and the 5 isolates of Klebsiella spp showed 100% sensitivity to Gentamicin & Ciprofloxacin.All the gram negative bacilli were resistant to Cotrimoxazole.

All the gram positive isolates like Staphylococcus aureus, Coagulase negative staphylococcus and Enterococci were 100% sensitive to Vancomycin. Gentamicin at 60% sensitivity was the second most effective drug followed by Ampicillin & Ciprofloxacin at 40% each.

Among the anaerobes, Metronidazole, Imepenum and Chlorumphenicol showed 100% sensitivity. The 7 isolates Bacteroides fragilis showed 100% sensitivity to Chlorumphenicol, Imepenum & Metronidazole and 71.4% sensitivity to Cetazidime clavulanic combination while one isolate of Peptostreptococcus anaerobius showed sensitivity to all the drugs namely, Penicillin, Vancomycin, Ciprofloxacin, Amikacin, Ceftriaxone, Clindamycin, Imepenum, Clhorumphenicol & Metronidazole.

DISCUSSION:

Bile represents a major challenge to the survival and subsequent colonization of microorganisms in the gastrointestinal tract. It is evident that certain bacteria have evolved to resist its actions and pathogens can even use bile to their advantage to regulate virulence determinants. Undoubtedly, knowledge gained through bile research will provide further insight into the survival of pathogens in vivo. An understanding of how bacteria can tolerate the high levels of bile encountered in the gallbladder may also explain persistent/chronic infections such as those caused by S. typhi. It is possible that information obtained from bile research may be exploited in antibacterial and preventive therapies.[17]

In recent decades, the antimicrobial resistance of bacteria isolated from bile samples of gall bladder disease has also increased. This necessitates a periodic monitoring of bacterial species and their antibiogram. Thus, the data presented in this study can provide information of immediate public health importance to clinicians on the selection of antimicrobial agents for the treatment of patients suffering from gall bladder diseases.

Most of the patients undergoing cholecystectomy were in the mean age group of 31-40 years, with ages ranging between 15 to 77 years, and this is consistent with the observations made by N.C. Dey (1968) .[18] Studies have revealed that bactobilia and post operative septic complications showed statistically significant correlation with high patient age. [19] The female to male ratio was 3.5:1. Similar observations were made by John T. Gowitz (1974).[20]

Maximum cases belonged to the cholelithiasis group. Studies have shown that when gallstone disease is present in Europe or America there are bacteria in bile in up to a third of the patients while up to two-thirds show the presence of bacteria in or intimately associated with the gallbladder wall.[21,22] Matin (1989) observed that a higher incidence of positive culture was shown in calcium bilitubinate and lower incidence was shown in cholesterol stones which suggests that bacteria in bile may play a role in the formation of pigment biliary stone.[23]

Pain abdomen was the commonest symptom.The associated risk factors jaundice, >60 years,obesity , diabetes mellitus ,choledocholithiasis . Antimicrobial prophylaxis should be given when one or more of these risk factors are identified pre-operatively. [24] Bhansali SK (1980) revealed that in acute cholecystitis, icterus may result due to presence of oedematous Hartman's pouch or pressure of stone impacted in Hartman's pouch on the choledochus.[25]

The primary finding of the study was that the overall prevalence of viable bacteria within bile sampled at cholecystectomy was 48%. Escherichia coli and Klebsiella spp. were found to be

commonest organisms in our study as already been reported elsewhere.[26,27] The importance of the predominance of E.coli is seen by the fact that glucosanidase enzymatic activity by E.coli may have a role to play in calcium bilirubinate gall stone formation.[28] However, this type of gall stone is uncommon in India.[29,30] Among the anaerobes Bacteroides fragilis was the commonest isolate followed by Peptostreptococcus anaerobius . studies also reported that the commonest isolate Bacteroides followed by Clostridium perfringes . [31] Mixed isolate of aerobe & anaerobe were Escherichia coli & Bacteroides fragilis, Klebsiella spp & Bacteroides fragilis. Animal model of biliary tract infection demonstrated that a combined B.fragilis & E.coli infection leads to a greater mortality and to a more frequent occurrence of liver abscesses than a purely aerobic or purely anaerobic infection.[32]

Gentamicin was found to be the most effective antimicrobial followed by by Ciprofloxacin , Ampicillin, and Ofloxacin.Darko R(1994) recommended Gentamicin for use as prophylaxis in biliary tract surgery. [5]Among the anaerobic isolates, Metronidazole, Chlorumphenicol and Imepenum were sensitive at 100%.

Thus, our bacteriological findings shows the presence of both aerobes and anaerobes with a ratio of 2.5:1. So immediate gram stain of bile at operation and bacteriological examinations for both aerobes and anaerobes of the patients with biliary tract infections is necessary, so as to analyse and compare the changing trend in the microbial etiology and their antibiogram pattern. Thus it will help to formulate a proper and appropriate antibiotics used against pathogens, to reduce likelihood of complications like wound infections and gram negative septicemia.

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