A MICROBIAL PROFILE OF PATIENTS WITH CHOLECYSTITIS AND/OR CHOLELITHIASIS UNDERGOING CHOLECYSTECTOMY ALONG WITH THEIR ANTIBIOGRAM

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ABSTRACT

BACKGROUND

Biliary tract disease is common cause of bacteraemia, associated with high morbidity and mortality. Most common infecting organisms are from Enterobacteriaceae family. Complications of bacteraemia are acute renal failure & septic shock.

MATERIALS AND METHODS

The study was conducted on 50 cases of cholelithiasis (who were admitted and treated in different units of General Surgery Department) and had undergone open or laparoscopic cholecystectomy. The samples collected were blood or bile, and then Gallstone Culture, Antibiogram Testing, Biochemical test (indole, methyl red, etc.) were performed.

RESULTS

Gallstones disease was more common in females as compared to males (F:M:3.5: 1) and the maximum age incidence was in 41-50 years age group (38%). Pain abdomen was the predominant symptom present in 88% of cases, flatulent dyspepsia in 44% cases and nausea/vomiting in 32% cases, while tenderness in right hypochondrium was present in 44% of cases. Out of total 50 cases, 18 (36%) showed positive bile cultures and incidence of positive cultures increased with advancing age with maximum being positive in 41-60 years age group. Gallstone culture was positive in 28 cases (56%). Most common organism isolated from bile was E. coli (44% of positive cases) followed by Klebsiella (22%) and Pseudomonas (16%).

CONCLUSIONS

Infection plays a key role in pathogenesis of chronic calculous cholecystitis and bile and gallstone culture is a good method to demonstrate the infective potential of bacteria colonising the gall bladder.

KEYWORDS

Biliary Tract Disease, Bacteraemia, Cholelithiasis, Open or laparoscopic Cholecystectomy, Gallstone Culture, Antibiogram Testing.

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BACKGROUND

Gallbladder disease has been recognised for nearly 1500 years and remains a major world health problem.

It is one of the most common disorders affecting the gastrointestinal tract and is an important cause of morbidity. At least three hundred million people inhabiting our planet are suffering from cholelithiasis. The incidence being 11-36% of the population.[1]

Cholecystitis and cholelithiasis with its complications 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 disease of the biliary tract and of these 1/5th or 3 million undergo biliary tract operations every year.

This disease is rare in the first two decades. Incidence gradually increases after 21 years and reaches its peak in the fifth and sixth decades.[2]

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Women are three times more likely to develop gallstone disease than men and first degree relatives of patients with cholelithiasis have a twofold greater prevalence of this disease.[1]

Gallstone disease is common and costly with over seven lakh cholecystectomies annually. In developed countries at least 10% of adults have cholesterol gallstones. Women have twice the risk and age further increases the prevalence in both sexes.

In India, gall bladder disease is much more common in North India than in South India. Similarly the incidence is higher in Eastern India than Western India.[3]

Different reasons for biliary tract infection have been presented, e.g. ascending infection due to reflex of duodenal contents, blood borne infection and infection spread through the portal venous channels. Ascending infection from the duodenum is thought to be the primary mechanism by which bacteria enter the bile. Different microbes in the bile may be the cause to post-cholecystectomy infections. Thus, understanding the most common organisms causing them and their antibacterial susceptibility pattern would be useful in prevention of these infections.[4]

Biliary tract disease is a common cause of bacteraemia and is associated with high morbidity and mortality, particularly in older patients with comorbid disease or when there is a delay

in diagnosis and treatment. The most common infecting organisms are Enterobacteriaceae ascending from the gastrointestinal tract.[5,6,7]

In patients with bacteraemia, complications such as acute renal failure and septic shock occur more commonly, so an improved understanding of the causative organisms, susceptibility profiles results in better clinical outcomes.[8]

The microorganisms predominantly found are Gram-negative aerobes like E. coli, Klebsiella, Proteus and Pseudomonas; Gram-positive cocci are also present like the Streptococci, Enterococci and Staphylococci. Anaerobes found in the bile are anaerobic streptococci, Clostridium welchii and Bacteroides fragilis.[9]

In Saudi Arabia, 25% of patients undergoing cholecystectomy for gallstones were bacterial culture positive and the most common organisms isolated were E. coli (28.1%), E. faecalis (15.6%) and P. aeruginosa (9.4%).[10]

The choice of antibiotics in patients with biliary sepsis will depend upon two important considerations- sensitivity and the concentration of antibiotics in bile. However, in patients undergoing elective surgery, the role of a prophylactic antibiotic is to achieve adequate serum levels above the minimal inhibitory concentration of the most likely suspected organism. Timing of antibiotic administration is crucial to prevent post-operative wound infections. Adequate tissue concentration of an antibiotic is necessary to achieve a maximal benefit.[11]

In spite of modern standards of pre-operative preparation and operative techniques, post-operative wound infections occur in quite number of patients. In spite of using higher antibiotics, wounds which are expected to heal by first intention do not heal as desired.[12]

Several authors have found the definite correlation between the species of bacteria cultured from bile collected during surgery both from gall bladder and common bile duct and the species cultured from post-operative infections. Thus, it is important to take bile cultures routinely at the time of operation for antimicrobial prophylaxis to reduce the post-operative infection rate. The incidence, types of bacteria involved and their susceptibility to antibiotics need to be accurately predicted on the basis of results from recent bacteriological investigations of bile.

This study was undertaken to determine the microbiological profile and antibiotic sensitivity pattern of microorganism isolated from blood, bile and gallstones in patients with cholecystitis with or without cholelithiasis undergoing cholecystectomy to decide whether there is a need for change in the standard empirical antibiotic therapy administered. The aim of this study was:

1. To calculate the positivity of patients with bacteraemia in biliary tract infections.
2. To study the microbial profile by culture of bile samples.
3. To analyse and identify the aetiological microorganisms causing bacteraemia/septicaemia by conventional blood culture techniques.
4. To isolate and identify organism causing formation of gallstones.
5. Finally to determine antibiotic sensitivity pattern/antibiogram of the above isolates.

MATERIALS AND METHODS

The present study was undertaken to determine the microbial flora in the blood, bile and gallstones in patients with cholecystitis with or without cholelithiasis. Fifty patients were included in this study which was conducted in the Department of Microbiology, J.L.N. Medical College, Ajmer for a period of one year.

Inclusion Criteria

1. Patients admitted in various surgical units of J.L.N. Hospital, Ajmer diagnosed with cholecystitis with or without cholelithiasis undergoing cholecystectomy.
2. Demographic profile: All age group and both sexes will be included.

Exclusion Criteria

1. Patients who have been already administered antibiotics.
2. Cases of malignancy of gall bladder or biliary tract.
3. Patients of hepatic failure and in hepatic coma.

Samples were processed as follows

Blood Culture

5-7 mL of blood in blood culture bottles containing brain heart infusion broth was taken using all aseptic precautions (Bedside inoculation).

Bile Culture

3 mL of bile collected during operation was inoculated in blood culture bottles containing brain heart infusion broth using all aseptic precautions.

Gallstone Culture

After cholecystectomy, the gallbladder was cut open and a sample of stone was removed. After surface decontamination with ethanol the stone was broken down and material from the core of stone was taken and crushed pieces of gallstones were aseptically inoculated in brain heart infusion broth.

- In the lab, the culture bottles were kept in incubator at 37^0C and were screened for any turbidity after 24 hrs. and after 48 hrs.
- In case the broth turned turbid, then subculturing was done on MacConkey agar and Blood agar which were then incubated at 37^0 C and then inspected for the type of growth.
- Colony characteristics of growth were noted.
- Then, Gram staining was done on the growth obtained on MacConkey or blood agar to identify the organisms.
- Then, biochemical tests were done from the growth to confirm identification of organisms.
- Hanging drop preparation for motility in Gram-negative bacteria for identification.
- Antibiotic susceptibility was done using the Kirby Bauer disc diffusion method using Muller Hinton agar for commonly used antibiotics depending on the growth i.e. Gram positive or Gram negative.
- Then, biochemical test result and Antibiotic sensitivity was recorded and analysed.

Gram-negative Bacilli

For Lactose fermenting and non-lactose fermenting colonies, further Gram staining for grouping, Hanging drop for Motility,

Catalase and Oxidase tests were done. After which the following biochemical tests were done. They include:

- Indole test
- Methyl red test
- Simmon's Citrate test
- Christensen's Urease test
- Phenylalanine deaminase test
- Triple sugar iron test
- Glucose fermentation
- Lactose fermentation
- Sucrose fermentation.

These tests were done for Gram-negative bacteria.

Gram-positive Cocci

For Gram-positive cocci – Catalase test, oxidase tests were done. For cocci in clusters which are oxidase negative & catalase positive--slide and tube coagulase, Mannitol fermentation tests were done.

For Gram-positive cocci in pairs and short chains which are catalase negative – Bacitracin sensitivity was done on BA.

Antibiogram Testing

The antibiogram testing was done as per CLSI guidelines using modified Kirby-Bauer method. If the organisms were not sensitive to any of the drugs, then a second line of antibiotics was put up using the same procedure as above.

Sl.No.	First Line Drug	Second Line Drug
1	Erythromycin (E) 15 µg	Amoxyclav (AMC) 20+10 µg
2	Co-trimoxazole (COT) 23.75+1.25 µg	Linezolid (LZ) 30 µg
3	Gentamycin (GEN) 10 µg	Vancomycin (V) 30 µg
4	Ciprofloxacin (CIP) 5 µg	
5	Oxacillin (OX) 1 µg	
Antibiotic Disc used for Gram-positive Organisms		

Sl. No.	First Line Drug	Second Line Drug
1	Tetracycline (TE) 30 µg	Amikacin (AK) 30 µg
2	Ciprofloxacin (CIP) 5 µg	Amoxyclav (AMC) 20+10 µg
3	Cefotaxime (CTX) 30 µg	Ceftazidime (CAZ) 30 µg
4	------	Netilmicin (NET) 30 µg
5	-------	Imipenem (IMP) 10 µg
Antibiotic Disc used for Gram-negative Organisms		

Sl. No.	First Line Drug	Second Line Drug
1	Tetracycline (TE) 30 µg	Amikacin (AK) 30 µg
2	Ciprofloxacin (CIP) 5 µg	Amoxyclav (AMC) 20+10 µg
3	Cefotaxime (CTX) 30 µg	Ceftazidime (CAZ) 30 µg
4	Piperacillin (PI) 100 µg	Imipenem (IMP) 10 µg
Antibiotic Disc used for Pseudomonas Species		

Based on these tests, the following individual organisms were identified:

Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli.

RESULTS

The present study was done to evaluate the antimicrobial profile and susceptibility pattern of the patients with cholecystitis with cholelithiasis undergoing cholecystectomy.

Gallstones disease was more common in females as compared to males (F: M: 3.5: 1) and the maximum age incidence was in 41-50 years age group (38%). This disease was more common in non- vegetarians as compared to the vegetarians (52% v/s 48%). Majority of patients were obese with 76% of patients having weight >50 kg.

Pain abdomen was the predominant symptom present in 88% of cases, flatulent dyspepsia in 44% cases and nausea/vomiting in 32% cases, while tenderness in right hypochondrium was present in 44% of cases. Out of total 50 cases, 18 i.e. 36% showed positive bile cultures and incidence of positive cultures increased with advancing age with maximum being positive in 41-60 years age group.

Gallstone culture was positive in 28 cases i.e. 56% of the total samples. Preoperative blood cultures of all patients were found sterile.

Most common organism isolated from bile was E. coli (44% of positive cases) followed by Klebsiella (22%) and Pseudomonas (16%).

E. coli was again the most common organism isolated from gallstone culture (43% of positive cases) but Pseudomonas was more commonly isolated from gallstones than Klebsiella (29% vs. 18%).

Regarding the antibiotic susceptibility of bile and gallstones, the isolates were highly sensitive to 3rd generation cephalosporins (ceftriaxone, ceftazidime) and aminoglycosides (amikacin).

Gram-negative isolates in bile were 100% sensitive to imipenem followed by 93% to Amikacin and Ceftazidime Clav (ceftazidime + clavulanic acid) and 85% to Amoxyclav (amoxicillin + clavulanic acid).

Staphylococcus was sensitive to Amoxyclav, Ciprofloxacin, Cotrimoxazole, Gentamicin, Linezolid, Tetracycline and Vancomycin but resistant to Ampicillin, Oxacillin and Erythromycin.

Enterococcus was sensitive to Amoxyclav, Ciprofloxacin, Linezolid, Tetracycline and Vancomycin but resistant to Ampicillin, Cotrimoxazole, Gentamicin and Erythromycin.

Age Group (yrs.)	Female	Male	Total	Percentage
11-20	-	-	-	-
21-30	5	2	7	14
31-40	11	5	16	32
41-50	16	3	19	38
51-60	3	-	3	6
61-70	2	-	2	4
71-60	2	1	3	6
Total	**39**	**11**	**50**	**100**
Table 1. Age and Sex Distributions				

Weight (Kg)	No. of Cases	Percentage
31-40	2	4
41-50	10	20
51-60	23	46
>60	15	30
Total	**50**	**100**

Table 2. Relationship with Weight

Sl. No.	Symptoms	No. of Cases	Percentage
1	Pain right hypochondrium	40	80
2	Diffuse pain abdomen	4	8
3	Nausea/Vomiting	16	32
4	Flatulent dyspepsia	22	44
5	Fever	6	12
6	Tenderness in right hypochondrium	22	44
7	Palpable gallbladder	4	8

Table 3. Sign and Symptoms

Bile Culture	No. of Cases	Percentage
Positive	18	36
Sterile	32	64
Total	**50**	**100**

Table 4. Bile Culture

Gallstone Culture	No. of Cases	Percentage
Positive	28	55
Sterile	22	44
Total	**50**	**100**

Table 5. Gallstone Culture

Organism	No. of Cases	Percentage
E. coli	8	44
Klebsiella	4	22
Pseudomonas	3	16
Enterobacter	1	6
Enterococcus	1	6
Staph. aureus	1	6
Total	**18**	**100**

Table 6. Organisms Isolated in Bile

Organism	No. of Cases	Percentage
E. coli	12	43
Klebsiella	5	18
Pseudomonas	8	29
Enterobacter	2	7
Citrobacter	1	3
Total	**28**	**100**

Table 7. Organisms Isolated In Gallstones

Antibiotics	Total No. of Gram –ve Isolates	No. of Organism Sensitive Cases	Percentage
Ampicillin	13	3	23.07
Amoxyclav	13	11	84.61
Amikacin	13	12	92.3
Ceftriaxone	13	9	69.23
Cefotaxime	13	10	76.92
Ceftazidime	13	10	76.92
Ceftazidime Clav	13	12	92.3
Ciprofloxacin	13	9	69.23
Gentamicin	13	10	76.92
Tetracycline	13	7	53.04
Imipenem	13	13	100

Table 8. Antibiotic Sensitivity of Bile Culture Isolates

Antibiotics	Total No. of Gram –ve Isolates	No. of Organism Sensitive Cases	Percentage
Ampicillin	19	2	10.52
Amoxyclav	19	15	78.94
Amikacin	19	17	89.47
Ceftriaxone	19	16	84.21
Cefotaxime	19	15	78.94
Ceftazidime	19	15	78.94
Ceftazidime Clav	19	17	89.47
Ciprofloxacin	19	12	63.15
Gentamicin	19	15	78.94
Tetracycline	19	10	52.63
Imipenem	19	19	100

Table 9. Antibiotic Sensitivity of Gallstone Isolates

DISCUSSION

In our study, maximum number of patients were in 41-50 years age group (19 cases-38%) and then in the 31-40 years age group (32%) and Female: Male ratio was 3.5:1.

These results are in contrast to the study done by Mohan H et al[2] in 2005 on 1100 cases in North India. They studied cases ranging from 10 years to 90 years and concluded that maximum number of patients were in the age group 31-40 years and the Female: Male ratio in their study was 6.4 :1.

A similar study done by Chaudhry et al[13] (1987) also reported maximum incidence in 31-40 years age group with a Female: Male ratio of 17:1. These results of this study are also in contrast with our study.

Our results are comparable with the study of Sutor D.J. et al[14] (1973) who also reported the maximum incidence to be in the 41-50 years age group with a Female: Male ratio of 3:1. Similarly, Vijaypal et al[15] (1980) had also reported that maximum number of cases were in the 4th and 5th decade and that a Female: Male ratio in their study was 2.4:1 which is comparable with our results.

Our study showed a slightly higher incidence of cholelithiasis in non-vegetarians than in vegetarians (54% of total cases vs. 46% of cases) which may be attributed to higher amount of saturated fatty acids in non-vegetarian diet.

Tsai C. J. et al. (2004)[16] were also of the same view and they in their studies concluded that higher consumption of saturated fatty acids is associated with increased risk of gallstone formation.

In our study, the maximum number of patients were in 51-60 kg weight group (46%) while 30% had weight >60 kg.

These results are comparable with Whiting M.J. et al (1984)[17] who also concluded that obese persons were at higher risk of cholelithiasis as they have increased biliary cholesterol levels.

Michael F. et al (1998)[18] were also of the same opinion and they reported that reduced physical activity and increased obesity are independent risk factors for symptomatic gallstone disease.

The pathophysiological role of bacteria in the formation of gallstones was proposed long ago and using molecular genetic techniques, bacteria or their components can be demonstrated in most gallstones. Apart from molecular techniques, many studies have identified bacteria using the classical culture methods.

In our study, we cultured blood, bile and material from the centre of gallstones to identify presence of infection in these samples and its possible role in causing disease and its complication.

Long ago Harding A.J.[19] in 1962 had shown the presence of microorganisms in culture of gallstones and similarly Large A. M.[20] in 1963 had also concluded that infection of bile caused change in permeability of gall bladder mucosa leading to cholelithiasis.

In our study, bile cultures were positive in 36% of cases and our results are comparable with other studies.

Z. I. Malik[21] in 2009 had reported a bile culture positive rate of 32% which is very similar to our study results, but Al. Harbi et al[10] (2001) had reported bile culture positivity of 25% which is lower than our results.

In our study, the highest incidence of bile infection was among 40-60 years age group (9 positive cases out of total 22 cases in that age group i.e. 41%). These findings are similar to PRL Gomes series (2006)[22] where they concluded that incidence of infection was 39% in patients >50 years of age.

In our study, the most common organism isolated in bile was E. coli (44% of positive cases) followed by Klebsiella (22% of positive cases), Pseudomonas (16%) and Enterobacter, Enterococcus and Staphylococcus each in 6% of positive cases. PRL Gomes et al. (2006)[22] had also found out similar organisms in their series and percentage of E. coli and Klebsiella were almost similar to our series (i.e. 40% and 35% respectively) but they reported Enterobacter in 20% and Pseudomonas in 5% which is different from our results.

In another study, Z.I. Malik[21] in 2009 had reported E. coli to be the commonest organism isolated in bile (44%). In contrast to our study, they did not isolate Klebsiella in any of their samples while they isolated Proteus in 25% of their culture positive case which was not isolated in any sample of our series.

Salmonella typhi has long been recognised as an important organism which is cultured from bile and has been considered as an important organism responsible for gallstone disease, but in our study we did not isolate Salmonella typhi in any of the samples.

In other studies (PRL Gomes et al,[23] Abeysuriya et al,[24] Z. I. Malik et al[21]), they also did not isolate Salmonella in any of their samples.

Organism	Our Series	PRL Gomes Series	Z.I. Malik Series	Abeysuriya Series
E. coli	44%	40%	44%	55.3%
Klebsiella	22%	35%	---	5.3%
Pseudomonas	16%	5%	12.5%	23.7%
Enterobacter	6%	20%	---	---
Enterococcus	6%	---	---	13.1%
Staph. aureus	6%	5%	5%	---
Salmonella typhi	----	---	---	---

Comparison of Organisms Isolated in our Study with Others

Our results are comparable with other series as far as the most common organism is considered i.e. E. coli, regarding other organisms the results are variable in different series, may be because of the different geographical locations of studies conducted.

In our study of the total 50 gallstones that were analysed by culture, 28 stones (56%) had positive growth, 22 i.e. 44% were sterile.

Our study was similar to the study of H.S. Shukla et al[25] in Banaras (2004) in that we could also isolate live bacteria from gallstone but our culture positive rates were low as compared to their study.

H.S. Shukla et al (2004) had reported that out of total 85 cases of cholelithiasis which they studied, 54 samples were positive, 12 were sterile and 19 samples were contaminated. While considering the organism isolated from gallstones the results of our study were different from H.S. Shukla series (2004)

We observed E. coli to be the commonest organism (12 cases out of 28 i.e. 43%) while Shukla et al had observed Klebsiella spp. to be commonest isolate (17.5 of their cases) while in our series Klebsiella was seen in 5 samples i.e. 18% of the cases. H.S. Shukla et al[25] reported E. coli in 15% of their cases. Our study showed Pseudomonas in 29% while it was 8.1% in Shukla series. Enterobacter and Citrobacter in our series were 7% and 3% while they were 7.5% and 2.5% respectively in H.S. Shukla series.

Salmonella typhi long thought to be responsible for gallstone disease was not isolated in our series but H.S. Shukla et al had reported it in 1.5% cases. Thus, the organisms and their relative percentages were quite different in our series.

Thus, in our study, infection was present in >50% of the cases either in bile or the stones or both; however, there was no isolate found in any of blood sample of patients because of chronic nature of infection after subsidence of acute illness. Hence, bacterial infection is an important contributory factor in pathogenesis of gallbladder disease.

Regarding the antibiotic susceptibility of the isolated organism from bile and gallstones our study concluded that among the 3rd generation cephalosporins, cefotaxime had low sensitivity, ceftriaxone had good sensitivity (80-85%) while ceftazidime had the best sensitivity result (90%). Among the aminoglycosides, amikacin had good sensitivity (varying up to 90%) while gentamycin had a poor sensitivity pattern.

Ampicillin had the lowest sensitivity rates (40%), ciprofloxacin had good sensitivity results (80-85%) while imipenem had 100% sensitivity rate.

Among the 3rd generation cephalosporins, our results are comparable with other series. Ceftriaxone had a good sensitivity pattern in our series as well as other series.

Cefotaxime showed low sensitivity pattern in our series while it had good sensitivity in PRL Gomes and Abeysuriya series. Amikacin and ciprofloxacin had high susceptibility rates in our series as well as in other series.

Gentamycin had low susceptibility rates in our series which is comparable with D. Valceanu series while the other 2 series had very good susceptibility rates for this antibiotic.

Antibiotic	Our series Bile Stone	PRL Gomes Series Bile+gallstone	D. Valceanu Series (Bile)	V.Abeysuriya Series (Bile)
Ceftriaxone	85 80	100	85	98
Cefotaxime	79 68	100	--	100
Ceftazidime	95 93	--	85	98
Amikacin	85 90	100	--	100
Gentamycin	70 65	100	74	100
Ciprofloxacin	85 90	88	--	100
Ampicillin	45 40	25	3.7	100
Imipenem	100 100	100	--	100
Comparison of Antibiotic Susceptibility (%) with other Series				

Thus, a 3rd generation cephalosporin with an aminoglycoside is a good empirical therapy to start with in cases of uncomplicated cholelithiasis and if patients are allergic to cephalosporin then ciprofloxacin may be used.

Our results thus comply with the previous studies regarding the presence of bacterial pathogen in the gall bladder of patients even while lacking any evidence of infection and inflammation, thus further enhancing their potent pathogenic implications and post-operative squeals that can occur following gallstones lost in peritoneal cavity during cholecystectomy.

CONCLUSION

Thus, we conclude that infection plays a key role in pathogenesis of chronic calculous cholecystitis and bile and gallstone culture is a good method to demonstrate the infective potential of bacteria colonising the gall bladder. Our study has shown bacteria in core of gallstones in 56% of cases, thus we recommend complete retrieval of all the stones and debris during cholecystectomy. Our study also recommends for an adequate antibiotic prophylaxis for cholecystectomy cases and a 3rd generation cephalosporin combined with an aminoglycoside is a good empirical therapy to start with.

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