INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 12, No. 1, p. 1-7, 2021

Open Access RESEARCH PAPER

A cross sectional study on Bacteriological profile and antibiogram of cholecystitis and cholelithiasis

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Keywords: Cholecystitis, Cholelithiasis, Bile culture, Bacteriological profile, Antimicrobial susceptibility testing

Publication date: January 30, 2021

Abstract

Cholecystitis is the most common disease of gastro intestinal tract contributing for 10% disease burden. Most of the time it is infective in origin. In view of the emerging multi drug resistance organisms, there is a need for guidance in empirical antimicrobial therapy in every clinical setting. To study the bacteriological profile and their antimicrobial susceptibility pattern in cholecystitis and cholelithiasis patients. A cross sectional study was conducted at Mamatha General Hospital, Kammam over a period of 2 years from September 2010 to September 2012. A total number of 62 clinically diagnosed cases of cholecystitis and cholelithiasis subjected to elective cholecystectomy were included in the study. Bile and gall stone samples were collected and processed aerobically, anaerobically according to standard microbiological techniques. Antimicrobial susceptibility testing was performed on all isolates by Kirby-bauer disc diffusion method and susceptibility pattern were recorded. A total number of 62 cases of cholecystitis were included in the study shows female preponderance of disease. Maximum number of cases belongs to 41 to 50 years age group. Out of 62 patients 62 bile samples and 58 gall stones specimen were collected and analyzed. Bile culture was positive in 24 cholelithiasis cases (41.37%), Gallstone culture was positive in 9 cases (15.51%). The two bile samples yielded anaerobic growth. Escherichia coli was the predominant organism in both samples. Bacterial isolates showing maximum susceptibility to ampicillin-sulbactum (100%), amikacin (80%). To optimized empirical antimicrobial therapy in cholecystitis and cholelithiasis patients prior knowledge of the prevalence of various bacteria and their antimicrobial susceptibility pattern in is required.

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Introduction

Cholecystitis and cholelithiasis is the most common disease of gastro intestinal tract contributing for 10% disease burden in western population and 17% in Asian population[1], which may vary between 11-36% depending on the geographical distribution of the population. The most common complication of cholelithiasis is chronic cholecystitis. Most of the time it is infective in origin. The reason behind the infection is either by ascending infection due to reflux of duodenal contents or infection spreading through the portal venous channels [2].

The microbial etiology of cholecystitis is mainly contributed by various bacteria like Escherichia coli, Klebsiella pneumoniae, Enterococcus, Proteus and Salmonella Typhi [3]. Few fungi like Candida, Non albicans Candida and moulds like Aspergillus are also associated with cholecystitis [4].

The data on bacterial etiology and antimicrobial resistance pattern of cholecystitis is limited. In view of the emergence of multi drug resistance organism and changing trends in bacteriological spectrum, there is a need for guidance in empirical antimicrobial therapy in every clinical setting [5]. This will help in treatment options there by allowing optimized antimicrobial therapy. Formulation of empirical antibiotic treatment guidelines is usually based on the prior knowledge of the prevalence of various bacteria and their antimicrobial susceptibility pattern in cholecystitis and cholelithiasis patients.

The present study is aimed to find the bacteriological profile and their antimicrobial susceptibility pattern in cholecystitis and cholelithiasis patients attending a tertiary care hospital.

Materials and methods

A cross sectional study was conducted in The Department of General Surgery, Mamatha General Hospital, Kammam over a period of 2 years from September 2010 to September 2012.

A total number of 62 clinically diagnosed cases of cholecystitis and cholelithiasis who were subjected to elective cholecystectomy were included in the study. Patients with immunosuppressive conditions, undergoing emergency surgery, Chronic liver disease with deranged liver function were excluded from the study. Patient demographic details and clinical history was recorded using a well formed questioner.

Specimen collection

1. Bile: At the time of surgery, 3-5ml of bile was collected from gall bladder through 10ml disposable syringe using all aseptic measures and placed in a sterile container.
2. Gall stones: Gall bladder was removed and opened. The stones were collected in a sterile container under aseptic conditions. The stones were separated into two groups: (1) Black stones and both black and brown stones were regarded as pigment stones, (2) Yellow stones were regarded as cholesterol stones. Biochemical analysis of stones was not carried out.

The specimens were labelled and sent to laboratory without delay for culture.

Microbiological processing

Direct microscopic examination of the bile was performed using Gram stain technique. The smears were examined for pus cells and microorganism. About 3ml of bile was inoculated into bile broth, blood agar and Mac Conkey agar plates. Gall stones obtained were washed with sterile normal saline. The stones were crushed and inoculated into bile broth, blood agar, and Mac Conkey agar plates.

The inoculated plates were incubated at 37ºC over a period of 24 to 48 hours under aerobic conditions. The plates were examined for bacterial growth. In case of bacterial growth the isolated organism was identified phenotypically using Gram stain, culture characteristics and various biochemical tests according to standard protocol.

After isolation of the organism antibiotic susceptibility testing was performed on all isolates by Kirby - Bauer disc diffusion method. For Gram positive organism amoxyclav, amikacin, levofloxacin, cefotoxin, and vancomycin were tested. For Gram negative organism ampicillin-sulbactum, amikacin, levofloxacin, cefuroxime, cefoperazone, cefipime, cotrimoxazole were tested. Sensitivity and resistance patterns were interpreted and recorded by measuring zone of inhibition in reference to CLSI Guidelines.

For anaerobic growth, bile samples and crushed gall stones were inoculated into thioglycollate medium and Robertson's cooked meat medium and incubated at 37ºC. The culture media were examined at 48 and 72 hours for bacterial growth. In case of turbidity, the samples were inoculated onto special anaerobic blood agar plates. The plates were then placed in an anaerobic jar (gas pack method) and incubated at 37ºC for 48 hours. Bacterial growth was identified by cultural characteristics, pigmentation, Gram staining and motility.

Results

A total number of 62 cases of cholecystitis were included in the study. Out of 62 cases 58 were presented with cholelithiasis and 4 were presented with acalculus cholecystitis.

Out of 62 cases 44(71%) were females and 18(29%) were males showing the diseases predominance in females.

Maximum number of cases belongs to 41 to 50 years age group comprising upto 32.25% (n=20), followed by 31 to 40 years age group comprising upto 22.58% (n=14).

When it comes to clinical presentation almost all patients were presented with pain abdomen as the chief complaint specifically in right hypochondriac region. In addition to that fatty food intolerance was present in 38 cases (61.29%), nausea in 18 cases (29.03%), vomiting in 14 cases (22.58%), dyspepsia was present in 13 cases (20.96%) and fever was the presenting symptom in 20 cases (32.25%). (Fig. 1) On examination Murphy's sign was positive in 40 cases (64.51%).

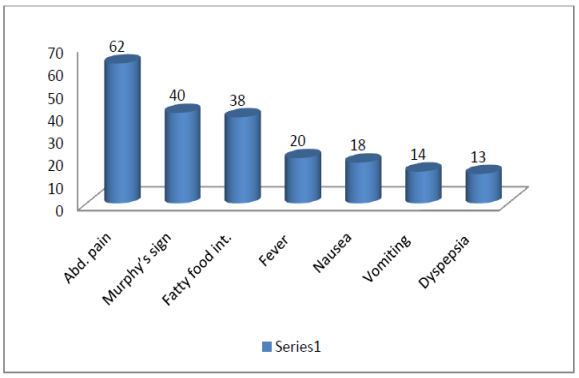

Fig. 1. Distribution of signs and symptoms.

Out of 62 patients 62 bile samples and 58 gall stones specimen were collected and analyzed. Out of 58 gall stones specimen 30(51.72%) were pigmented and 28(48.27%) were cholesterol stones.

Bile culture was positive in 24 cholelithiasis cases (41.37%), Gallstone culture was positive in 9 cases (15.51%). Out of the 9 culture positive gall stone samples 7(23.33%) were from pigmented stones and 2(7.14%) were from cholesterol stones. No growth was isolated in all the 4 acalculus cholecystitis cases. (Fig. 2)

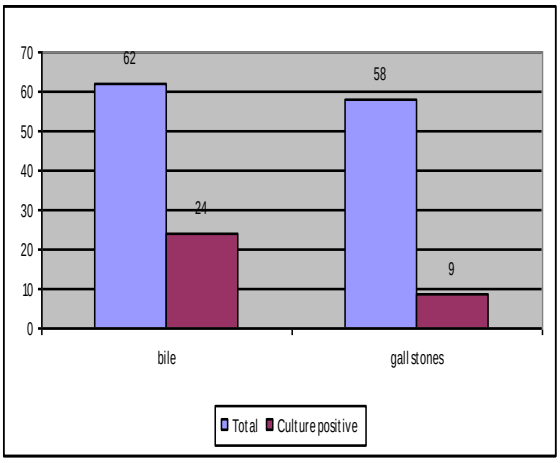

Fig. 2. Culture positivity.

Out of 24 isolates of bile culture 22 were aerobic organism and 2 were anaerobic organism, out of 9 isolates of gall stone culture all were aerobic organism. All the aerobic isolates from the gallstone cultures were similar to the isolates in bile cultures from the same patients.

Analysis of bacterial flora shows Escherichia coli is the predominant organism in both bile samples and gall stone samples comprising up to 50% (n=12) , 77% (n=7) respectively of all isolates.

The other organism isolated in bile samples were Klebsiella species (n=3), Pseudomonas aeruginosa (n=2), Staphylococcus aureus (n=2), Acinetobacter baumannii (n= 2), Citrobacter koseri (n=1). The two anaerobic isolates were Peptostreptococci species (n=1), Bacteroides fragilis (n=1). The other organism in gall stones were Klebsiella species (n=1), Staphylococcus aureus (n=1).

Gram negative organism showing maximum susceptibility to ampicillin- sulbactum (100%), amikacin (80%). Followed by levofloxacin (75%), cefipime (75%), cotrimoxazole (50%) were tested.

All Gram positive organism showing sensitivity to vancomycin (100%), and amoxyclav (100%), followed by amikacin (80%), levofloxacin (80%). None of the Staphylococcus aureus isolates showed resistance to Cefoxitin.

Discussion

The estimated prevalence of gallstone disease in India has been reported as 2% to 29% [6,7]. In India, this disease is seven times more common in the North (stone belt) than in South India[8].

In the present study bile samples and gallstone specimens were collected from diagnosed cases of cholecystitis and cholelithiasis. Bile samples and gall stones were collected at the time of surgery and processed within one hour of collection.

A total of 62 bile samples and 58 gall stones were collected and processed further. Bacterial cause was identified in 24(38%) bile samples and 9(15%) gallstone specimens. In Marne C *et al.*[9] study, bile cultures were positive in 70(56%) out of 125 patients.

In the present study the age of the patients was ranging from 21 to 70 yrs. More number of cases were found in the age group of 31–50 yrs in our study. In Suri A *et al.,* study[3], conducted at Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu, also shows that more number of cases belong to 31 – 50 yrs. age group. In Mohan H *et al.,* study[10], the maximum number of patients were in the age group of 31 – 40 yrs (316 cases, 28.7%). In R.G. Willis and W.C. Lawson series[11], more number of cases are found in the age group of 51 – 60 yrs. The average age of these patients in India, is a decade younger than those in the West[12]. In our study, the mean age is 43.08 and SD ±12.4. Majority of patients were in the age group of 31–50 yrs.

In the present study, male to female ratio was 1:2.5. In Ohood Akead study[13], the male to female ratio was also 1:2.5. In Abd-Alkareem study[14], male to female ratio was 1:2.8. In Suri A *et al.,*[3] study, male to female ratio was 1:3 In Nasir Mahmud Wattoo[2] study, male to female ratio was 1:3.5. In almost all the studies, female ratio was more than males.

In the present study the patients with varied signs and symptoms were nearly consistent with the signs and symptoms of Suri A *et al.,*[3] study.

In our study, there were 30 pigment stones and 28 cholesterol stones. In Jayanthi V *et al.,*[15] study and Tyagi SP *et al.,*[16] study also reported similar incidence where majority of gall stones are pigment stones and the remaining are cholesterol and mixed type. But in Mohan *et al.,*[17] study, 62.3%, 17.3%, 14.1%, and 3.2% were mixed, cholesterol, combined, and pigment

stones respectively. In our study, bile culture was positive in 21.42% of cholesterol stones and 60% of pigment stones. In Vasitha *et al.,*[4] study, culture was positive in 26% of cholesterol stones and 82% of pigment stones. 28 of the 38 bile samples were shown positive only after enrichment in BHI medium. Probably, this explains the difference in this study and our study. Pigment gall stones contain calcium palmitate, calcium bilirubinate and conjugated bilirubin which are associated with bile infection.

Of the 58 gall stones processed, 9 (15.51%) yielded growth of aerobic bacteria which were similar to the isolates in bile cultures from the same patients. In Ballal *et al.,*[18] study, 6(24%) yielded growth of aerobic bacteria out of 25 gall stones.

In our study, common organism isolated from bile was Escherichia coli in 12 cases (50%), followed by Klebsiella (12.5%), Pseudomonas, Staphylococcus aureus and Acinetobacter (8.33%) and Citrobacter (4.16%). This is nearly consistent with Ballal M *et al.,*[18] study. In Vasitha *et al.,*[4] study, the common organism isolated was Escherichia coli (51.7%).

In Abd-Alkareem[19] study, common organism isolated was Escherichia coli (48%). In Zafar Iqbal Malik *et al.,*[20] study, the common organism isolated was *Escherichia coli* (43.75%). In almost all the studies, the common organism isolated was Escherichia coli. In Dhir *et al.,*[21] study, Pseudomonas has been reported as the predominant flora.

In the present study only 2(8.33%) out of 24 bile samples were positive for anaerobic bacteria. Peptostreptococci species (4.16%) and *Bacteroides fragilis* (4.16%) were isolated and species identification was done by cultural characteristics and Gram staining. In Ballal M *et al.,*[18] study, anaerobes isolated were Peptostreptococci (14.28%) and *Bacteroides fragilis* (42.85%). In Suri A *et al.,*[3] study, anaerobes were not identified in any patient. In Bergan T[22] study, bacteriological examination was made of bile from 119 patients with cholecystitis and/or cholelithiasis. In an initial series of 50 samples from the gall bladder cultivated aerobically and anaerobically in thioglycollate broth, only aerobes were isolated from 48% of the patients. In a second series of 69 patients, the anaerobic techniques were more adequate: sampling by puncture with oxygen exclusion, microbiological processing within 10 minutes and use of anaerobically stored media. The results of aerobic cultivation were similar in the two series, but in the latter 18% of the bacterial strains were anaerobes alone or in combination with aerobes. The anaerobes were fairly equally distributed between the genera Bacteroides, Clostridium and Peptostreptococcus (two, three and four isolates respectively).

In our study, aerobic sensitivity to Gram negative organism showing maximum susceptibility to ampicillin- sulbactum (100%), amikacin (80%). This is consistent with Suri A *et al.,*[3] study. The resistance to cephalosporins has increased while Betalactum and Betalactamase inhibitor combinations were better sensitive and can be used as the first line of treatment. The next most sensitive drug was Amikacin. This is consistent with PRL Gomes *et al.,*[23] study.

Conclusion

The results of this study depict a clear association between bile infection and pathogenesis of gall stones as patients without gall stones did not have bacteria in their bile. Antibiotic sensitivity patterns of isolated organisms were similar irrespective of the type of stone. Bile infection is a potential risk factor in gall stones formation and bile culture is a must. But the problem lies in collection of bile from the operation theatre and inoculation within an hour of collection.

We have shown that the importance of obtaining cultures of the bile at the time of cholecystectomy lies in the fact that appropriate

antibiotics can be administered in the event of a positive culture to forestall serious complications like gram negative septicaemia.

It is concluded that the culture of the organism from the bile at the time of the operation does not necessarily indicate a cause-effect relationship between the infective microorganism and lithogenesis, as infection may be secondary to calculus formation. The failure to isolate organism from bile also does not indicate that the etiology is unrelated to the infection as it is well known that organism which have initiated the stone precipitation may not persist in the viable form in the bile till surgery.

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