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BACTERIAL ANALYSIS OF BILE IN CHOLECYSTECTOMY PATIENTS

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ABSTRACT

Background: Acute inflammation of the gallbladder wall usually follows obstruction of the cystic duct by a stone. Bacterial inflammation which may play a role in 50% to 85% of patients of acute cholecystitis. The common organisms isolated from bile culture in this patients are Esch. Coli, Klebsiella spp, Streptococcus spp, B.fragilis and clostridium species. **Aim:** The purpose of this study is to isolate and identify the bacterial infections of the bile in gallbladder diseases at RIMS Hospital, Imphal and to know the drug sensitivity pattern of the isolated bacteria. **Material and Method:** This study was done in the Department of Microbiology in collaboration with the Department of Surgery RIMS, Imphal on patients undergoing elective cholecystectomy for gall bladder diseases during the period from October 2020 to October 2022. **Results and Discussion:** Out of the 131 samples collected, culture was positive in 22 while the total number of isolates were 25 as three cases had coinfection with 2 isolates each. Gram negative bacteria with 17 isolates (68%) were much more common than the Gram positive bacteria with 8 isolates (32%). The commonest organism was Escherichia coli 9/25 (36%), followed by Klebsiella pneumoniae 5/25 (20%), Staphylococcus aureus 4/25 (16%), Enterococcus faecalis 4/25 (16%) and Enterobacter spp 3/25 (12%). The Gram negative bacteria was 100% sensitive to Colistin and imipenem, 88% sensitive to gentamicin, 35.2% sensitive to ceftazidime and 58.8% to a combination of ceftazidime and clavulanic acid. Extended spectrum β-lactamase production was seen in 44.4% of Escherichia coli, 20% in Klebsiella pneumoniae and 33.3% of Enterobacter spp. Among the Staphylococcus aureus, 100% sensitivity was seen to linezolid, vancomycin and gentamicin, 75% sensitivity to erythromycin and ciprofloxacin, 50% sensitive to clindamycin, 100% resistance to ampicillin and 75% resistance to amoxicillin was seen. One methicillin resistant Staphylococcus aureus was isolated. The Enterococcus faecalis were 100% sensitive to vancomycin and linezolid. **Conclusion:** routine bile culture and sensitivity should be done in patients undergoing cholecystectomy as it will guide the surgeons for empirical antibiotic therapy, antibiotic prophylaxis and pathogens directed therapy to reduce the clinical outcome indicators such as morbidity and mortalility and to prevent emergence of drug resistance.

INTRODUCTION

over 95% of the biliary tract disease is attributed to cholelithiasis (gall stones). gallstones are classified into cholesterol or pigment stones but the majority of the stones are of the mixed type. the majority of the stones in the developed nations are of the cholesterol type while in the developing countries the pigment stones are more common. 90% of the stones are of the cholesterol type.1

acute inflammation of the gallbladder wall usually follows obstruction of the cystic duct by a stone. inflammatory factors can be evoked by three factors: 1) mechanical inflammation produced by increased intra-luminal pressure and distension with resulting ischemia of the gallbladder mucosa and wall. 2) chemical inflammation caused by the release of lysolecithin and the other local tissue factors. 3) bacterial inflammation which

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may play a role in 50% to 85% of patients of acute cholecystitis. the common organisms isolated from culture of bile in these patients are escherichia coli, klebsiella spp, streptococcus spp, bacteroides fragilis and clostridium spp.2 acalculous or non-calculous cholecystitis account for 10 % of biliary symptoms shown to have chronic inflammation within the gall bladder but an absence of gallstones. 3 cholecystectomy is the most cost effective and the only reliable method and it remains the treatment of choice. other modes of treatment are medical treatment, gallstone dissolution and extracorporeal shock wave lithotripsy and mechanical litholysis.4 pain in the epigastrium and the right hypochondrium, occurring 30−60 minutes after meals is frequently associated with gallstone disease. diagnosis of cholecystitis is made by the presence of biliary colic with evidence of gallstones on an imaging study. ultrasonography is the diagnostic test of choice, being 90−95% sensitive. the surgical literature suggests that 3−10% of patients undergoing cholecystectomy will have cbd stones.5

the purpose of this study is to isolate and identify the bacterial infections of the bile in gallbladder diseases at rims hospital, imphal and to know the drug sensitivity pattern of the isolated bacteria.

Aims And Objects

1. to isolate and identify the bacteria causing infections of gall bladder diseases in patients undergoing cholecystectomy in rims hospital imphal.
2. to study the drug sensitivity pattern of the isolated bacteria to the commonly used antibiotics.

MATERIALS AND METHODS

Sample Size: a total of 131 samples were collected for the study. the sample size was calculated as 131 with an error margin of 8% taking the prevalence as 30%.6 bile samples were taken from patients undergoing cholecystectomy in the department of surgery rims hospital.

Duration of Study: october 2020 to october 2022.

Place of Study: department of microbiology, rims in collaboration with the department of surgery, rims, imphal.

Collection of Specimen: 5- 10 ml of bile, pus or other exudates was aspirated in a sterile needle and syringe7 under aseptic precautions, from the gall bladder during cholecystectomy. 2-3 ml of bile was inoculated immediately into robertson's cooked meat medium. the remaining sample was transferred into sterile scintillation vial. samples were immediately transported to the department of microbiology, rims for processing.

Processing of Specimens: specimens were subjected to the following

- Direct smear was made, methanol fixed and gram stained.
- The samples were inoculated onto glucose broth, selenite f broth, blood agar and macconkey agar medium
- Inoculated media then incubated at 37oc for 24 to 48 hrs
- The colony morphology was studied and recorded
- On the second day sub-culture was done from selenite f broth to macconkey agar, and from glucose broth onto blood agar and macconkey agar.
- The colonies were then subjected to a series of tests for identification of the isolates as per standard bacteriological identification guideline 8, 9.
- Antibiotic susceptibility was carried out by kirby bauer disc diffusion method in accordance with latest csli guidelines.10 ethical approval was obtained from the institutional ethical committee, rims, imphal before the beginning of the study.

RESULTS AND OBSERVATION

a total of 131 samples of bile from patients undergoing cholecystectomy for gallbladder diseases were included in the study. the data collected were then analysed.

Age and sex distribution: out of 131 patients who underwent cholecystectomy for gallbladder diseases, the maximum number of patients was in the age group of 31-40 years (28.3%) closely followed by the age group of 21-30 years (24.4%). the minimum number of patients was seen in the age group of 71-80 years. the females (85.5%) outnumbered the males (14.5%) in all age groups. the male to female ratio was 1: 5.8. the patients were in the age group 18 to 75 years.

Table1. Age and sex distribution

Age in years	Male		Female	
	number	Culture +ve	number	Culture +ve
<20	01	0	05	1(20.0%)
21-30	05	1 (20.0%)	27	4 (14.8%)
31-40	08	1 (12.5%)	29	3 (10.3%)
41-50	03	1 (33.3%)	22	4 (18.9%)
51-60	03	1 (33.3%)	17	5 (29.4%)
61-70	02	0	06	0
70 -80	00	0	03	1 (33.3%)
Total	22	4 (18.9%)	109	18 (16.5%)

Diet wise distribution: majority of the patients were non-vegetarians with 112 patients (93.1%) and 6.1% were vegetarians.

CULTURE RESULTS

Of the 131 patients, 22 (16.8%) were culture positive, while total bacterial isolates were 25 as 3 cultures were polymicrobial. gram negative bacteria constituting 17/25 (68%) while the gram positive isolates were 8/25 (32%). escherichia coli (36%) was the commonest organism, followed by klebsiella pneumoniae (20%), staphylococcus aureus and enterococcus faecalis 16% each. the enterobacter spp was the least common isolate seen (12%). co-infection was seen in 3 (2.3%), 2 were males with co-infection with escherichia coli and klebsiella pneumoniae and a female patient had coinfection with staphylococcus aureus and enterobacter faecalis. among the male patients with co-infection one patient had a history of gastro-jejunostomy 10 years back and the other male patient was a diabetic.Culture positivity and post-operative hospital stay: the post-operative hospital stay of the patients ranged from 3 to 65 days. majority of the patients got discharged by the 5th day.

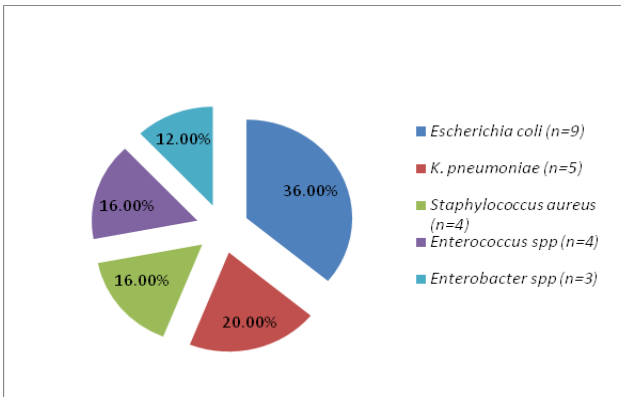

Figure 1. Different bacterial isolates recovered from bile.

The culture positivity had no relation with the post operative hospital stay, 86.7% of the culture positive patients were also discharged by the 5th day.

Table 2. Culture positivity and post-operative hospital stay

post-operative stay in hospital (days)	Total patients	Culture positive pts	
0-5	86 (65.6%)	19	P-value 0.261 DF-4
6-10	35 (26.7%)	02	
11-15	05 (3.8%)	01	
16-20	04 (3.1%)	00	
21 <	01 (0.7%)	00	

antibiotic sensitivity pattern of gram negative bacteria: all the gram negative bacteria were 100% sensitive to colistin and imipenem, 88% sensitive to gentamycin, 35.2% sensitive ceftazidime and 58.8% to combination of ceftazidime and clavulanic acid as shown in figure 4. among the gram negative bacteria extended spectrum β-lactamase production was seen in 6 isolates, 44.4% (4/9) escherichia coli, 20% (1/5), klebsiella pneumoniae and 33.3% (1/3) enterobacter spp.

Table 3. antibiotic sensitivity pattern of gram negative bacteria

Antibiotic	*E. coli* (n=9)	*K. pneumoniae* (n=5)	*Enterobacter* spp (n=3)
Gentamicin	8 (88.80%)	4 (80%)	3 (100%)
Ampicillin	1 (10.20%)	1 (20%)	0 (00%)
Ampicillin + Sulbactam	2 (22.20%)	1 (20%)	1 (33.30%)
Ceftazidime	4 (44.40%)	1 (20%)	1 (33.30%)
Ceftazidime + Clavulanic acid	7 (77.70%)	1 (20%)	2 (66.70%)
Ceftriaxone	6 (66.70%)	2 (40%)	1 (33.30%)
Imipenem	9 (100%)	5 (100%)	3 (100%)
Ofloxacin	6 (66.70%)	3 (60%)	2 (66.70%)
Colistin	9(100%)	5(100%)	3(100%)

antibiotic sensitivity pattern of staphylococcus aureus:
among the 4 isolates of staphylococcus aureus, methicillin resistance was seen in 1 (25%) isolate. staphylococcus aureus isolates were 100% sensitive to vancomycin, linezolid and gentamicin, 75% sensitive to erythromycin and ciprofloxacin, 100% resistant to ampicillin and 75% resistant to amoxiclav.

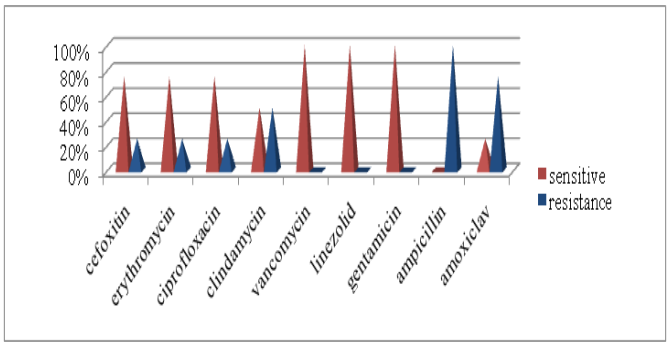

Figure 2. antibiotic sensitivity pattern of staphylococcus aureus.

Antibiotic susceptibility pattern of enterococcus faecalis: the enterococcus faeccalis were 100% sensitive to vancomycin and linezolid, 75% sensitive to high level gentamicin, 50% sensitive to ofloxacin, ampicillin and amoxiclavulanic acid combination.

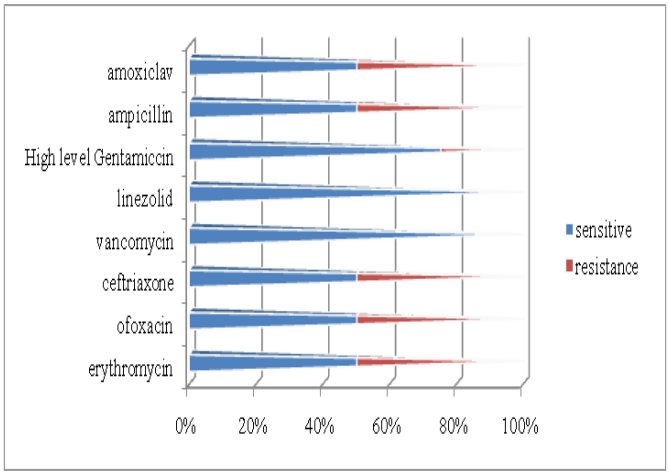

Figure 3. Antibiotic susceptibility pattern of enterococcus faecalis

DISCUSSION

The study was designed to provide information on the bacteriological profile and antimicrobial patterns of the isolated bacteria from bile in patients with gallbladder diseases.

the age of the patients in this study ranged from 18 to 75 years. the maximum group of patients were in the age group 31-40 years (28.25%) similar to a study done in kathmandu, nepal where the most commonly involved age group for cholelithiasis (32.5%) was in the age group 30-39 years.11 it was closely followed by 24.43% in the age group 21-30 years. similarly, it was found that females especially multipara were frequently affected; majority of cases were in 3rd to 5th decade of their life, rise in age showing decline in incidence.12 other studies have shown higher incidence of cholelithiasis among younger age group of 20-30 years. in a retrospective study, maskey cp et al 13 also found similar findings that the commonest age group for cholelithiasis was below 30 years comprising 37.5%.

the females out-numbered the males in all the age groups, 22 (16.8%) of the patients were males and 109 (83.2%) females, the male to female ratio being 1:5.8. a study done in chandigarh, india14 where they reported the male to female ratio as 1:1.7. a geographical variation were present in sex incidence of the disease, the females had a preponderance over males in the north with female: male ratio 2:1, in the south the males showed a preponderance with an inverse female: male ration of 1:3. 15

non-vegetarians (93.1%) were found to be more commonly involved with gallstone diseases. the ratio of non-vegetarians to vegetarians was 1:12.4. the exact cause cannot be stated however, it could be due to the consumption of high protein and fat. in a similar study done by kotwal mr et al16 in sikkim and north bengal, india, 97% cases of cholelithiasis were found in non-vegetarians.

aerobes were identified in all the culture-positive patients, whereas no anaerobes were isolated in any patient, similar to the study conducted by hazra p et al.17 in this study the coliforms were the commonest with 68% followed by enerococcus faecalis and staphylococcus aureus 16% each similar to other studies.18 in this study escherichia coli 9/25 (36%) clearly dominated, klebsiella pneumoniae 5/25 (20%), staphylococcus aureus 4/25 (16%), enterococcus spp 4/25 (16%) and enterobacter spp 3/25 (12%). this is more or less similar with the study conducted by mohd talha noor et al.19, 20

in this study, the gram negative bacteria was 100% sensitive to colistin and imipenem, 88% sensitive to gentamicin, 35.2% sensitive to ceftazidime and 58.8% to a combination of ceftazidime and clavulanic acid. yadav et al21 conducted a similar studies in northern india, the gram negative bacteria was 100% sensitive to colistin, 88.7% sensitive to imipenem, 66.1% sensitive to gentamicin. among gram positive,this study shows 100% sensivity to vancomycin and linezolid in both staphylococcus aureus and enterococcus faecalis which shows similar pattern in the case of yadav et al21.

in this study there were 6 patients of choledocholithiasis with obstructive jaundice out of which 3 (50%) were culture positive and those with obstructive cholangitis, bile cultures were positive more frequently and more organisms were resistant to ampicillin and first generation cephalosporins.22

CONCLUSION

The culture positivity rate in the present study was 16.8% (22 cases). the total bacterial isolates were 25 as three of the cases had co-infection with 2 isolates each. 14.5% of the isolates were monomicrobial while 2.3% was polymicrobial. it is therefore suggested that routine bile culture and sensitivity should be done as far as practicable in patients undergoing cholecystectomy as it will guide the surgeons for empirical antibiotic therapy, antibiotic prophylaxis and pathogens directed therapy to reduce the clinical outcome indicators such as morbidity and mortalility and to prevent emergence of drug resistance.

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