

Bacterial Profile of Bile and Gall Stone in Symptomatic Cholelithiasis Patients

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ABSTRACT

Purpose: The aim of this study to identify the microbial profile in bile and gallstone of patients undergoing cholecystectomy and antibiotic susceptibility pattern of the isolates.

Introduction: Cholelithiasis is one of the most common disease affecting gastrointestinal tract and common indication for abdominal surgeries.

Materials and Methods: The retrospective study was conducted at Department of Microbiology, Rajendra Institute of Medical Sciences (RIMS), Ranchi from November 2015 to October 2016. Cholelithiasis diagnosed patients admitted to Department of surgery; RIMS, Ranchi for open and laparoscopic cholecystectomy were taken for study. The total numbers of patients were 257. Bile aspirated from gallbladder and gall stones in sterile container containing normal saline was brought to the Department of Microbiology for culture and sensitivity test.

Results: On culture of bile and gall stone, the most common bacteria was *E. coli* followed by *Klebsiella*. Both organisms isolated from culture showed maximum sensitivity to Amoxicillin/Clavulanic acid, Piperacillin / Tazobactam. *E. coli* showed maximum resistance to Trimethoprim –

Sulphamethoxazole while *Klebsiella* showed maximum resistance to Ciprofloxacin.

Conclusion: Culture of bacteria from bile need to be necessary to prevent complication after cholecystectomy.

Keywords: Cholelithiasis, Culture and Sensitivity Test, Antibiotics.

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INTRODUCTION

Gallbladder disease has a worldwide variation, being common in US, where an estimated 15 million people have gallstone and of these 1/5th or 3 million undergoes biliary tract operation every year.¹

Gall stone disease is common problem in the elderly women and there has been well known association of this disease with obesity and multiparity. This disease has been found very infrequently in children.² Incidence of gallstones increases with the age. It is more common in female than male (4:1) and about 50% patients are asymptomatic.³ The pathogenesis of gall stone is multifactorial. Primarily gallstone can be divided into two major groups. First is pure gallstone contributing 10% of gall stones; second is mixed and combined stone which account for 90% of gallstone. Mixed stone frequently associated with cholelithiasis.⁴ The causation of gall stones is associated with different factors amongst which infection of the bile is also as important factor.

Inflamed gallbladder has markedly altered permeability, which permits absorption of bile acids and movement of inorganic salts into the gallbladder lumen. The role of excessive cellular debris and increased protein secretion, which occurs in response to inflammation, may be present. Finally, bacterial enzymes effects constituency of bile which may alter its solubility leading to precipitation of bile.⁵

The most common reason for a cholecystectomy is cholelithiasis. Cholecystectomy is currently a frequently performed operation. However, the presence of gallstones within either the gallbladder or biliary tree is associated with the bacterial colonization in the bile.⁶

Therefore, it is necessary to know the microbiological flora of the bile before prophylactic antibiotics to be given. Prophylaxis would be appropriate according to bacteria isolated from the bile and could prevent postoperative infections.

MATERIAL AND METHODS

Study Design, Period and Area

This was a prospective observational study done in patients undergoing cholecystectomy for cholelithiasis which included all age group patients ranging from 9 years to 82 years. The study was conducted between October 2015 to September 2016 in Department of Microbiology at Rajendra Institute of Medical Sciences (RIMS), Ranchi, Jharkhand, India.

Study Subjects

The patients were admitted a night before surgery in the concerned department and were examined and investigated by concerned surgical team and anaesthetic team for fitness for surgery. Consent was taken from the patient or patient's attendant after explaining the nature of procedure and potential for conversion from laparoscopic approach to open cholecystectomy. All of these patient had preoperative diagnosis of cholelithiasis and were operated either laparoscopically or conventional open technique. All patients were given an IV injection of Ceftriaxone 1 gram at induction of anaesthesia and 2 doses of the same were repeated postoperatively.

Sample Collection, Handling and Transport

Intra-operatively the abdomen was examined, findings recorded. Gall bladder was removed after ligation and cutting of the cystic artery and duct. The bile aspirated from gallbladder and the gall stone was collected in a sterile container containing normal saline and labelled and it was sent to Department of Microbiology, RIMS, Ranchi immediately.

Culture and Identification

The gall stone and bile sample was incubated in Brain heart infusion (BHI) medium at 37°C for 24 hours. Then on next day, sample was inoculated on blood agar and MacConkey agar medium and incubated at 37°C for 24 hours. The gall stone was sterile when there was no growth even after 48 hours of aerobic incubation. All positive culture was further identified by their characteristic appearance on the media; microscopic examination of Gram's staining; motility testing in gram negative bacilli by hanging drop method and confirmed by the pattern of biochemical tests using the standard procedures. Once detected the antimicrobial sensitivity of these bacteria were done.

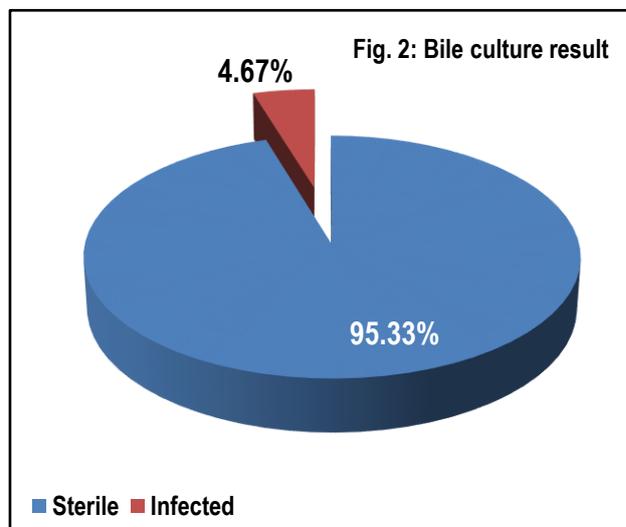
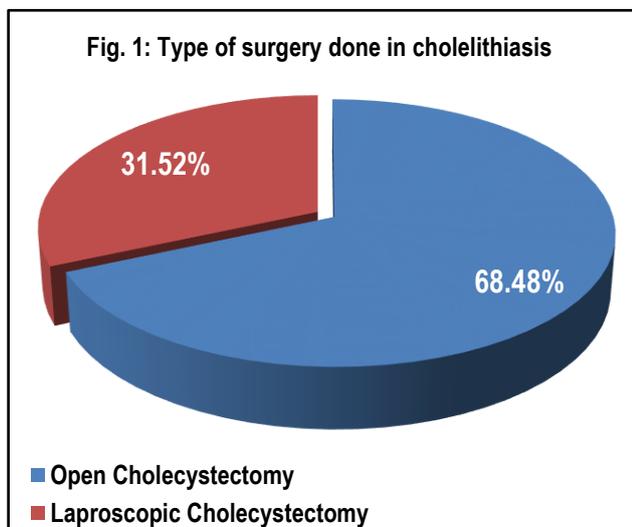


Table 1: Organism isolated from bile culture

Organism isolated	No. Of positive bile culture	Percentage
Escherichia coli	7	58.33
Klebsiella pneumoniae	3	25
E. coli + Klebsiella	2	16.67
Total	12	100

Table 2: organism isolated from gall stone culture

Organism isolated	Positive gall stone culture	Percentage
Escherichia Coli	7	77.78
Klebsiella pneumonia	2	22.22

RESULTS

The total number of patients presenting with symptomatic cholelithiasis were 257. Maximum patient were found in age group of 31-40 years. Out of these male and female patient were 53 (20.47%) and 204 (79.53%) respectively with male to female ratio of 1: 3.85. In male patients maximum patients were found in age group of 41-50 years (26.42%) and in female patients maximum

patients were found in the age group of 31-40 years (29.41%). Out of 257, 176 cases undergone open cholecystectomy (68.48%) while 81 cases which undergone laparoscopic cholecystectomy (31.52%). (Figure 1) Out of 257, 245 (95.33%) bile samples were sterile. Only 12 (4.67%) bile samples were infected. (Figure 2) The most common isolated organism from bile culture was Escherichia coli (58.33 %) followed by Klebsiella were found in 25% of cases.

Polymicrobial infection (*Escherichia coli* + *Klebsiella*) found in 16.67%. (Table 1) The most common organism isolated from bile culture was *Escherichia coli* (77.78%), followed by *Klebsiella* which was present in 22.22 % of cases.(Table 2) In present study in 4 cases both bile culture and gall stone culture was positive. (Figure 3) Gall stone culture was positive in 7 case out of 136 cases of pigment stone (7.14%), 1 cases out of 98 case of cholesterol type (0.74%) and in 1 case of mixed stone of gall stone out of 23 (4.38%). (Table 3) In different type of gall stone, gallstone culture were positive in 9 cases out of 257 (3.50%) of which maximum were in the age group of 31-40 years (4.17%). Pigment stone were the most common type of gall stone found but maximum gall stone culture positive found in mixed type of gall stone. The most common organism isolated from gallstones was *Escherichia coli* (77.78%) followed by *klebsiella* which were present in 22.22% of cases. On bile culture and sensitivity test, *E. Coli* showed maximum sensitivity to Amoxicillin/Clavulanic acid, Pipracillin/Tazobactam, Imipenem, Amikacin in 9 (100 %) cases followed by Gentamicin in 8 (88.89%) cases. *E. coli* showed high

resistance to Ciprofloxacin, Ceftazidime in 6 (66.67%) cases followed by Ceftazidime in 4 (44.44%) cases. *Klebsiella* showed maximum sensitivity to Amoxicillin/Clavulanic acid, Pipracillin/Tazobactam, Imipenem, Amikacin in 5(100 %) cases followed by Gentamicin, Ceftriaxone, Ceftazidime (80%) cases. *Klebsiella* showed high resistance to Ciprofloxacin, Norfloxacin in 4 (80%) cases followed by Trimethoprim-Sulphamethoxazole, Nitrofurantoin in 3 (60%) cases.

On gallstone culture and sensitivity test, *E. Coli* showed maximum sensitivity to Amoxicillin/Clavulanic acid, Pipracillin/Tazobactam, Imipenem, Amikacin in 7 (100 %) cases followed by Gentamicin in 6(88.71%) cases. *E.coli* showed high resistance to Trimethoprim-Sulphamethoxazole in 1 (100%) cases followed by Ceftazidime, Nitrofurantoin in 3 (42.85%) cases. *Klebsiella* showed maximum sensitivity to Amoxicillin/Clavulanic acid, Pipracillin/Tazobactam, Gentamicin, Ceftriaxone, Ceftazidime, Imipenem, Amikacin in 2(100 %) cases followed by Nitrofurantoin in 1 (50%) cases. *Klebsiella* showed high resistance to Ciprofloxacin in 2 (100%) cases followed by Norfloxacin in 1 (50%) cases.

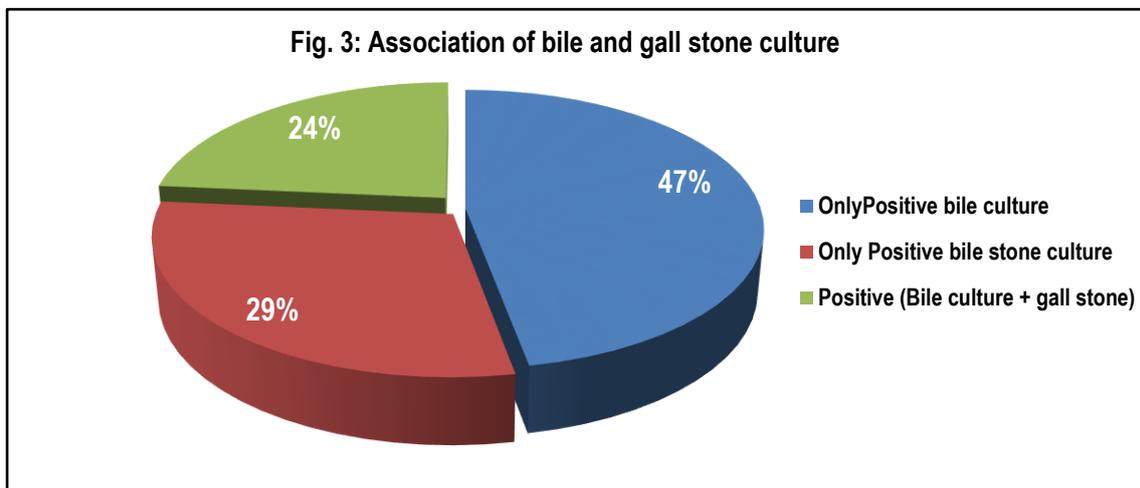


Table 3: Type of gall stone found

Type of gall stone	Total no. of cases	Positive gall stone culture	Percentage
Cholesterol	98	1	0.74
Pigment	136	7	7.14
Mixed	23	1	4.38

DISCUSSION

In our study we observed that, the maximum number of patients presenting with symptomatic cholelithiasis were 72 (28.03%) and belonged to the age group of 31 to 40 years followed by 62 (24.12%) between the age group of 41 to 50 years. Gall stone disease was more common in females with the patients belonging to the age group of 31 – 40 years (29.41%), whereas in males older age group was more common with patients belonging to the age group of 41 – 50 (26.42%). With increasing age, males tend to have decreased physical activity hence predisposing to gall stone formation. A study by Kalina Z et al (1992) also showed that prevalence of gall stone is higher in females and in both sexes, its incidence increases with age.⁷ Similarly, Channa et al (2004), reported that the peak age group for the occurrence of gall stones in males was between the age group 45 – 59 years and in females 30 -44 years which is consistent with our study.⁸

In our study, it was observed that the females were commonly affected by cholelithiasis and 79.53 % of patients were females with male to female ratio as 1: 3.85. Similar observation were found by various studies –Ahmad, et al (2014) and Manan, et al (2014) had recorded male to female ratio of 1: 3.85.^{9,10} while Mayank Devangan, et al (2015) and Ozydin, et al (2016) had recorded male to female ratio of 1: 2.9 and 1: 2.17 respectively.^{11,12}

In the present study, pigmented stones were predominant, which was found in 136 (52.92%) cases out of 257. Cholesterol stones were the second most common which was found in 98 (38.13%) cases. Mixed type of gall stones were found only in 23 (8.05%). Shaffer et al in (2006) stated that the most common type of stone in developed countries was of cholesterol type.¹³ A study by Chennai- Jayanthi v et al (1996) showed that the pigmented gall stones were most common (63.8%) followed by mixed stones

(34.8%) and then by cholesterol (1.9%).¹⁴ A study by Mayank Devangan et al (2015), pigment type of gallstones was most common type (65%).¹¹ Malatani Tarek et al (1996), showed that mixed gall stone are commonest type (58.5%), pigment stone (27%) and cholesterol stone (14.5%).¹⁵ A study by Abd-Alnabi et al (2014), stated that the commonest type of gall stone was cholesterol stone 36 (37%), followed by pigment stone 33 (34%), and mixed stone 26 (27%).¹⁶ Al-Khateji et al (2006) showed that the most common type of gall stone was of cholesterol type (40%) followed by pigment stone (35%) and mixed stone (25%).¹⁷

On correlating different type of stones with infection of bile, pigment stones association with infection (7/136) (7.14%) was more common than other stones followed by mixed stone (1/23) (4.38%) and cholesterol stone (1/98) (0.74%) in my study. A study by Shrestha K R et al (2014), Pigment stones are associated with infection of bile pigment stone were found to be more infected than other infection. In their study showed commonest organism in cholesterol stones as *E coli* (7/19) while in pigment stones commonest organism isolated was *K pneumoniae* (3/7) and in mixed stone multiple organisms were isolated (*E coli*, *K pneumoniae* and *Enterococcus spp.*).¹⁸ Mayank Devangan et al (2014), showed that gallstone culture was positive in 7 cases out of 27 cases of pigment stone (25.9%), 1 cases out of 21 case of cholesterol stone (9.5%), and in 2 cases of mixed type of gall stone out of 2.¹¹

On bile culture and sensitivity test, *Escherichia Coli* showed maximum sensitivity to Amoxicillin / Clavulanic acid, Piperacillin / Tazobactam, Imipenem, Amikacin in 9 (100 %) cases followed by Gentamycin in 8 (88.89%) cases. *Escherichia coli* showed high resistance to Ciprofloxacin, Ceftazidime in 6 (66.67%) cases followed by Ceftazidime, Trimethoprim-Sulphamethoxazole in 4 (44.44%) cases. *Klebsiella pneumoniae* showed maximum sensitivity to Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Imipenem and Amikacin in 5(100 %) cases followed by Gentamicin, Ceftriaxone, Ceftazidime in 4 (80%) cases. *Klebsiella* showed high resistance to Ciprofloxacin, Norfloxacin in 4 (80%) cases followed by Trimethoprim-Sulphamethoxazole Nitrofurantoin in 3 (60%) cases. On gallstone culture and sensitivity test, *E. coli* showed maximum sensitivity to Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Imipenem, Amikacin in 7(100 %) cases followed by Gentamicin in 6(88.71%) cases. *E.coli* showed high resistance to Trimethoprim-Sulphamethoxazole in 1 (100%) cases followed by Ceftazidime, Nitrofurantoin in 3 (42.85%) cases. *Klebsiella* showed maximum sensitivity to Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Gentamicin, Ceftriaxone, Ceftazidime, Imipenem, Amikacin in 2 (100%) cases followed by Nitrofurantoin in 1(50%) cases. *Klebsiella* showed high resistance to Ciprofloxacin in 2 (100%) cases followed by Norfloxacin in 1 (50%) cases.

Fazal Manan et al (2014), on culture and sensitivity test, *E. Coli* showed high sensitivity to Cefuroxime in 22 (78.57%) cases followed by Ceftriaxone in 21 (75.00%) patients. *E. coli* showed high resistance to Amoxicillin in 17 (60.71%) patients followed by resistance to Ciprofloxacin in 12 (42.86%) patients. *Klebsiella* showed high sensitivity to Ciprofloxacin in 13 (72.22%) patients. The resistance of *Klebsiella* was noted maximum to Amoxicillin which was in 10 (55.56%) patients.¹⁰ A study by Gomes PRL et al (2008), showed that hundred percent of coliforms were sensitive to carbapenem (imipenem), 95% to aminoglycoside (gentamicin

and amikacin), fluoroquinolone (ciprofloxacin), 81% to amoxiclav and ureidopenicillins (piperacillin). Third generation cephalosporin (cefotaxime- 100% and ceftriaxone-95%) were more effective against coliforms than second generation cephalosporin cefuroxime 86%. *Pseudomonas spp.* was sensitive to aminoglycosides, ciprofloxacin and piperacillin.¹⁹

CONCLUSION

Gall stone disease is major health problem throughout the world. The present study was conducted to study the role of bacteria in the lithogenesis of gallstone, for which both bile and gallstone were subjected for culture, as only bile culture really could not suggest the presence of bacteria in gallstone which may have initiated lithogenesis. So for this culture of organism from gallstone need to be necessary and correlating it with culture of microorganism isolated from bile. Apart from surgery prompt administration of appropriate antibiotics to control the biliary tract infection is also very important.

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