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RESEARCH ARTICLE

BACTERIOLOGICAL PROFILE OF BILE CULTURE AND SENSITIVITY PATTERN OF ITS ISOLATES IN ADULT PATIENT OF BILIARY TRACT INFECTION IN TERTIARY CARE HOSPITAL OF BIHAR

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ABSTRACT

Background and objectives: Bacterial infection of biliary tract may cause severe inflammatory response or sepsis. Cholecystitis and cholelithiasis are the most common diseases affecting the gastrointestinal tract. An immediate bile culture and appropriate antibiotic administration are important to control the biliary tract infection. The objective of the study was to study the microbial profile and antibiotic sensitivity pattern in patients with biliary tract infection.

This cross sectional descriptive study was conducted at Medicare gastro centre and Sahyog hospital, Patliputra, Patna, India for 12 months i.e., from September 2017 to August 2018. Total 100 cases were included in this study. For the prospective group, only patients who had not received antibiotics prior to endoscopic retrograde cholangiopancreatography (ERCP) and had given written informed consent were included.

Results: On culture and sensitivity test, 47% have positive growth while 53% have no growth. The most common bacteria was *Escherichia coli* isolated in 22 (51.16%) patients followed by *Klebsiella* 9 (20.93%), *Pseudomonas* 4 (9.30%) *Salmonella* and *Citrobacter* each 3(6.98%), and *Proteus* 2(4.65%). On sensitivity test, all the Gram negative bacilli were sensitive to Tigecycline followed by Colistin, Imipenem and Amikacin.

Conclusions: The most common bacteria of symptomatic cholelithiasis are *Escherichia coli* and *Klebsiella* followed by *Pseudomonas* and *Salmonella*. These bacteria showed maximum sensitive to Tigecycline, Colistin followed by Imipenem and Amikacin. Early detection and determination of antimicrobial susceptibility pattern is important to reduce the mortality and morbidity associated with bile fluid infections.

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INTRODUCTION

Biliary calculus disease is one of the most common disorder affecting gastrointestinal tract. There has been marked rise in the incidence of gallstone disease in the west during past century. In India, it is more common in the North India than in South India. Similarly the incidence in eastern India is higher than western India. Incidence of gallstones increases with age. It is more common in female than male M:F =1:4 and about 50% patient are asymptomatic (Ballal and Jyothi, 2001). Bactobilia are not found in healthy individuals, since daily excretion of bile helps to flush out whatever organisms enter the biliary tract, but the percentage of bactobilia increases to 3% in patients with gallstones and to 30% in patients with common duct stones (Kaufman, 1989). Gallstone disease is common worldwide, and its prevalence has geographical and ethnic variations. The lowest prevalence is seen in Africans (Lee et al., 2007; Shivaprakasha et al., 2006).

In the National Health and Nutrition Examination Survey III study, the overall prevalence of gallstone disease in the United States was 7.9% in men and 16.6% in women (Solomkin et al., 2002). Cholelithiasis is an important cause of morbidity throughout the world (Qureshi, 2006). Among different factors causing gallstones formation, biliary infection can be found in a sizeable proportion of patients. Gallstones cause various problems besides simple biliary colic and cholecystitis (Lorenz et al., 1998). Human bile though sterile normally, can become infected in biliary tract obstruction due to entry of microorganisms through various routes like papilla of Vater or hematogenous leading to bactobilia (Chang et al., 2002). The pathogenesis of bile infection is incompletely understood, with the prevailing theories not fully explaining all the observations (Bae et al., 2008; Kiesslich et al., 2001).

MATERIALS AND METHODS

The descriptive cross sectional study was carried out at Medicare gastro centre and Sahyog hospital, Patliputra, Patna,

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India for 12 months i.e., from September 2017 to August 2018. Total 100 cases were included in this study. For the prospective group, only patients who had not received antibiotics prior to endoscopic retrograde cholangiopancreatography (ERCP) and had given written informed consent were included. All patients with symptomatic cholelithiasis, 18 years or older of either gender were included in the study. The patients with Acute cholecystitis (severe right upper quadrant pain with pyrexia and leucocytosis; 12000-15000 cells/ μ L); Obstructive jaundice (raised alkaline phosphatase >two times upper limit of normal), Common bile duct stone stones (on Ultrasonography); already receiving antibiotics (from history), were excluded from the study as they were liable to produce bias in the study results. All the study patients presenting with symptoms (Pain right Hypochondrium, and Vomiting), and sign (Tender right Hypochondrium) were admitted through OPD.

The diagnosis was confirmed on ultrasonography (showing distended gall bladder with calculi). Routine investigation like Full blood count, blood urea and sugar, Serum electrolytes and investigations for an aesthesia fitness like chest X-ray, ECG and Liver function tests, Viral markers were performed. The purpose and procedure of the study were explained to the patients and a written informed consent was obtained. The collected specimen of the bile was collected in 5cc disposable syringe. For aerobic culture, the sample was inoculated on blood agar, Nutrient agar and Mac-Conkey agar medium and incubated at 37^C for 24-48 hours. Culture plates were incubated aerobically at 37⁰c for 24-48 hours. The isolates were identified by colony morphology, gram-staining and a battery of biochemical reactions. Antimicrobial susceptibility testing was performed on Mueller Hinton agar using disc diffusion method in accordance with Clinical and Laboratory Standard Institute Guidelines (CLSI). Zone size of each antimicrobial agent were recorded and interpreted as resistant, intermediate or susceptible. Intermediately susceptible isolates were considered resistant. Patient demographics like age, gender and culture reports of bile were recorded in a structured Performa.

RESULTS

The total number of patients presenting with symptomatic cholelithiasis were 100. Out of these, male and female patients were 27% and 73% respectively. The mean age of male and female patients with symptomatic cholelithiasis were 48.20 years and 46.25 years respectively. Growth was obtained in 43% bile samples. The organisms obtained were *Escherichia coli* isolated in 22 (51.16%) patients followed by *Klebseilla* 9 (20.93%), *Pseudomonas* 4 (9.30%) *Salmonella* and *Citrobacter* each 3(6.98%), and *Proteus* 2(4.65%). On sensitivity test, all the Gram negative bacilli were sensitive to Tigecycline followed by Colistin, Imipenem and Amikaci.

Table 1. Microbial profile of the isolates from bile sample: (N=43)

Isolates	Number	Percentage
<i>Escherichia coli</i>	22	51.16 %
<i>Klebsteilla pnemoniae</i>	09	20.93 %
<i>Pseudomonas aruginosa</i>	04	9.30 %
<i>Salmonella typhi</i>	03	6.98 %
<i>Citrobacter spp.</i>	03	6.98 %
<i>Proteus spp.</i>	02	4.65 %

Table 2. Antibiotic sensitivity pattern of Gram negative bacilli isolates (N=44)

Antibiotic disc	Sensitive No. (%)	Resistant No. (%)
Ampicillin (A)	5 (11.36%)	39(88.64%)
Amikacin (AK)	31 (70.45%)	13 (29.55%)
Ceftazidime (CAZ)	15 (34.09%)	29 (65.91%)
Ceftriaxone (CTX)	19 (43.18%)	25 (56.82%)
Ciprofloxacin (CIF)	21 (47.72%)	23 (52.28%)
Gentamicin (G)	28 (63.64%)	16 (36.36%)
Meropenem (MR)	32 (72.73%)	12 (27.27%)
Imipenem (IMP)	35 (79.55%)	9 (20.45%)
Piperacillin-Tazobactam (PTZ)	27 (61.36%)	17 (38.64%)
Colistin (CL)	41 (93.18%)	3 (6.82%)
Tigecycline (TG)	44 (100%)	0

DISCUSSION

Acute cholangitis is a life-threatening complication of bile duct obstruction requiring emergency care. Fever, jaundice and abdominal pain (Charcot's triad), the common symptoms seen in our patients are similar to other studies (Lee and Chung, 1997; Agarwal *et al.*, 2006). Male to female ratio in this study was 1:3, in all literature available so far females have been easiest victim of gallstone disease. In fact, female sex hormone and sedentary habits of most women in India expose them to factors that possibly promote the formation of gallstones (Zuhair *et al.*, 2011; Dhar *et al.*, 2001). Bile in normal patient is found to be sterile, however in about 30-40% of the patient with cholelithiasis bacteria can be cultured from the bile (Stewart *et al.*, 1987). In this study incidence of bile culture positivity was (43/100) 43%, and the most common organism isolated was *E.coli* followed by *Klebseilla*. In other study it is found that mixed stone is frequently associated with cholecystitis. In Ohdan H et al study incidence of positive bile culture was 38% and *E .Coli* being the common organism isolated (Ohdan *et al.*, 1993). *E.coli* was found to be the commonest organism in this study, and it also reported in previous studies. However *Klebsiella pneumoniae* was reported by Stewart *et al.* (44%), Balla et al (46%) (Ballal). The bacteriological profile of acute cholangitis has remained stable over the last three decades but their antibiotic susceptibility pattern has changed. Ampicillin with gentamicin was the agent of choice in the past. With increasing resistance to ampicillin and significant nephrotoxicity caused by aminoglycosides, this combination fell out of favour (Lee *et al.*, 1992; Kiesslich *et al.*, 2001). A recent report from India by Shivaprakasha and colleagues on biliary bacterial isolates from 128 samples showed high resistance of gram-negative bacilli (GNB) to ampicillin (92.4%), cephalixin (82.46%), ciprofloxacin (68.42%) and piperacillin (64.33%) (Shivaprakasha *et al.*, 2006) In our study antibiotic sensitivity patterns of isolates showed that high resistance to Ampicillin, Ciprofloxacin and third generation Cephalosporins. A high sensitivity of isolates to Colistin (93.18%), Tigecycline (100%) and Imipenem (79.55%) was observed in our study as documented in other studies (Shivaprakasha *et al.*, 2006; Kiesslich *et al.*, 2001; Lee *et al.*, 1992).

Conclusion

The most common bacteria of symptomatic cholelithiasis are *Escherichia coli* and *Klebseilla* followed by *Pseudomonas* and *Salmonella*. These bacteria showed maximum sensitive to Tigecycline, Colistin followed by Imipenem and Amikacin. Early detection and determination of antimicrobial

susceptibility pattern is important to reduce the mortality and morbidity associated with bile infections.

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