International Surgery Journal
Dewangan KP et al. Int Surg J. 2022 Sep;9(9):1577-1590
http://www.ijsurgery.com
pISSN 2349-3305 | eISSN 2349-2902

DOI: https://dx.doi.org/10.18203/2349-2902.isj20222235

Original Research Article

Microbiological profile of bile culture and its antimicrobial susceptibility pattern in endo-biliary stented patients undergoing cholecystectomy

Komal Prasad Dewangan[1,2]*, Srinivasa Rao Geddam[1,3], Partha Sarathi Nayak[4]

[1]Department of General Surgery, The Calcutta Research Institute, Kolkata, West Bengal, India
[2]Department of General Surgery, Visakha Institute of Medical Sciences, Visakhapatnam, Andhra Pradesh, India
[3]Department of General Surgery, Chhattisgarh Institute of Medical Sciences (CIMS), Bilaspur, Chhattisgarh, India
[4]Department of General Surgery, Ramakrishna Mission Seva Pratisthan and Vivekananda Institute of Medical Sciences, Kolkata, India

Received: 07 July 2022
Revised: 03 August 2022
Accepted: 06 August 2022

***Correspondence:**
Dr. Komal Prasad Dewangan,
E-mail: drkomaldewangan1986@gmail.com

ABSTRACT

Background: The vast majority of common bile duct (CBD) stones was secondary, formed within the gall bladder (GB) and migrates down the cystic duct into CBD or rarely due to primary stones formed in CBD. Intra-operative bile culture prevents development of infectious complications and guide selection of future appropriate antibiotic prophylaxis. Hence, this study focussed on investigation of microbiological profile of routine bile cultures in endo-biliary stenting patients undergoing cholecystectomy.
Methods: In this study, 50 patients with CBD stone(s) were undergone endoscopic retrograde cholangio-pancreatography (ERCP) followed by stenting and then cholecystectomy (either laparoscopically or open). GB extraction was done in a sterile bag and after opening sterile bag; bile was collected and sent for microbiological culture and sensitivity for analysis.
Results: Most of the cases had cholelithiasis with choledocholithiasis (38.0%) which were significantly higher than other diagnosis ($Z=2.51$; $p<0.05$) and there was no significant association found between surgical site infections and bile culture positivity of studied patients ($Z=9.61$; $p<0.0001$). Among 35 patients with positive bile cultures, *E. coli* in 26 patients followed by *Klebsiella species* in 5 patients were the most prevalent bacteria isolates and over all, colistin (91.4%) and tigecycline (91.4%) were found as sensitive antibiotics. Also, certain strains of multi drug resistance *E. coli* in 6 patients were resistant to gentamicin/amikacin only sensitive to tigecycline and colistin, which was high.
Conclusions: Most prevalent isolates microorganism is gram negative bacteria that were mostly gut bacteria and the incidence of post ERCP infection is high and drug resistance among the causative organism is common.

Keywords: Common bile duct stones, Gall bladder, Bile culture, Surgical site infections, Antibiotics

INTRODUCTION

The incidence of gallstone disease has increased significantly worldwide including India and constitutes a significant health problem in developed societies affecting 10-15% of the adult population.[1] Cholecystectomy is the treatment of choice in gallstones disease. A portion will present with CBD stones. The vast majorities of CBD stones are secondary, formed within the GB and migrate down the cystic duct into CBD or

rarely due to primary stones formed in CBD, mainly seen in benign biliary strictures, sclerosing cholangitis and choledochal cyst.[2]

The exact incidence and prevalence of CBD stones among patients with gallstone disease are not known but according to literature these CBD stones are found in range of 10-15% of patients having gallstones disease.[3] Factors associated with cholesterol stones include nutrition, obesity, increasing age, female sex, pariety.[3] Secondary choledocholithiasis may be asymptomatic or symptoms similar to those seen with gallstone disease.[2]

Choledocholithiasis complicates the work up and management of gallstone disease, necessitates additional expensive diagnostic and therapeutic procedures and adds to the morbidity and mortality of gallstone disease.

Clinical features of choledocholithiasis leads to symptoms include intermittent fever, pain in upper abdomen, fluctuating jaundice and complications that includes cholangitis, pancreatitis and sepsis.

Management of choledocholithiasis usually requires two separate teams: the gastroenterologist and the surgical team.[4] Diagnostic investigations include USG, MRCP for confirmation which is often followed by therapeutic option of pre-operative ERCP with ES, CBD clearance by extracting stones for choledocholithiasis and CBD stenting followed by cholecystectomy either open or laparoscopically.

Delay of cholecystectomy after doing ERCP with stenting leads to a higher incidence of infected bile.[5] Patients with infected bile have a tendency for developing biliary events during the waiting time for LC/OC.[6] Presence of bactibilia did not influence operating time and did not lead to more difficulty or complications.[7]

USG has low sensitivity, 25-60% in detecting CBD stone although it may detect dilatation of the CBD or the intrahepatic biliary radicals which are supportive evidence of biliary obstruction.[8] MRCP is a very good diagnostic tool with very high sensitivity; but is expensive and lacks any therapeutic advantage.[9] ERCP is good therapeutic tool to detect and extract CBD stones; with its own set of complications such as trauma, hemorrhage and cholangitis and pancreatitis.[10]

Routine use of antibiotic prophylaxis prior to ERCP to reduce the incidence of sepsis/cholangitis cannot be recommended as per meta-analysis.[11]

There is significant shift in ERCP towards therapeutic indications and a decline in its conventional diagnostic utility.[4] Several studies showed that patients with obstructed bile ducts are at highest risk of developing septic complications following ERCP, especially when the drainage was not complete.[12,13]

Bile is normally sterile. Bactibilia occurs when bile flow is impaired and bacteria gain access to the biliary duct by the papilla or portal circulation. Bactibilia is a predictor of septic complications following biliary tract surgeries. Bile-contaminated operations are reported to have a higher incidence of septic complications than non-bile contaminated operations.[14]

There is high correlation of post-operative septic complications in patients with positive bile culture with rates as high as 80-89%.[15-17] Post-operative wound infection in biliary surgery is caused by endogenous contamination due to opening of biliary tract in patient with bactibilia, both post operative wound infection and septicemia is mostly caused by same organism.[18]

Intraoperative bile culture result was thus a guide to early institution of appropriate antibiotic in patients at risk. This prevents development of infectious complications and guide selection of future appropriate antibiotic prophylaxis. Further, this information helps monitor cleansing and disinfection services in endoscopy unit and help formulate antibiotic policy for a hospital or region.

In infected bile, the typical pathogens are the gram-negative enteric aerobes such as *Escherichia coli*, *Klebsiella species* and *Proteus species*, while *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Enterococcus faecalis* are less commonly cultured.[19]

Prophylactic antibiotics prevent infections even though they do not sterilize bile rates of bactibilia are not reduced by prophylactic antibiotics that achieve bile levels that exceed the minimum inhibitory concentrations of recovered bacteria.[20]

This study was focussing towards the analysis of microbiological profile of routine bile cultures in endo biliary stenting patients undergoing cholecystectomy, many of whom were often asymptomatic. Also, in the past no study had been done in eastern part of India to access the culture and sensitivity of bacteria, if present in bile following stenting.

METHODS

Type of study

The study performed was a prospective, observational, single centre clinical study.

Study period

The study was conducted from July 2017 to May 2019.

Source of data

50 consecutive patients, undergone cholecystectomy in department of surgery, CMRI Hospital, Kolkata with patients had CBD clearance by ERCP with stented who

met the inclusion criteria had included in the study and their data were analyzed. Duplication of cases due to admission of same patient in various departments of the hospital was avoided by comparing the name, age, address and medical records departments/MRN numbers. Data was noted in the study proforma (appendix) and was tabulated using Microsoft Excel.

Inclusion criteria

Patients with CBD stent following clearance of CBD calculi through ERCP and requiring subsequent cholecystectomy; patients who required cholecystectomy and exploration of CBD after failed stone clearance following ERCP and stenting; patients with ERCP and stenting done in our tertiary care hospital were included.

Exclusion criteria

Patients with established case of cholangitis (as per Tokyo Guidelines, 2018 mentioned in methodology below); patients diagnosed with malignancy of gall bladder or CBD; patients who were given broad spectrum antibiotics after ERCP and stenting; patients who denied consent to participate in the study; patients who had bile spillage during cholecystectomy were excluded.

Sample size

According to Khusroo et al in a study titled "Prevalence of biliary tract disease in India" in 1988 showed prevalence of GB stone in India as 6.12%; according to Rai et al "Incidence of choledocholithiasis in gallstone disease in eastern zone of India" in 2016 showed prevalence of CBD stone as 18%.[21,22]

The formula used for sample size calculation was as follows,

$$n=\frac{\alpha^2 pq}{e^2},$$

where,

n=required sample size, p=proportion, q=1-p, e=precision.

Here α=5%,

Hence,

zα=1.96, p=18% of 6%=1.08%,

e=4%.

n was calculated to be 46.

Total sample size was calculated as 46, but we took it as 50.

Methodology

ERCP mainly had following steps: sphincterotomy; CBD stone(s) removal; stent placement (plastic).

Patient had not received any prophylactic antibiotics before ERCP with stenting procedure.[5] Patient also had not received antibiotics after ERCP followed by stenting, which was done in our tertiary care hospital, CMRI Kolkata, which was done with all aseptic precautions.

Followed by surgery

As per the hospital protocol, prophylactic antibiotic was given during induction followed by cholecystectomy/common bile duct exploration (laparoscopically) and gall bladder extraction in a sterile bag. With use of sterile syringe of 10 ml with 20 G needle, bile aspiration done in vitro by puncturing the wall of extracted GB and about 2 to 8 ml of bile was collected and transferred in a sterile container or tube and sent for microbiological culture and sensitivity in department of microbiology.

In case of open cholecystectomy, GB was taken out and kept in container and with the help of sterile syringe of 10 ml with 20 G needles, bile aspirated *in vitro* by puncturing the wall of extracted GB. Approximately 2 to 8 ml of bile (mean of 4 ml) was collected and transferred in a sterile tube and send for microbiological culture and sensitivity in department of microbiology.

Post cholecystectomy endo biliary stent was removed after 4 to 6 weeks of surgery, in cases where laparoscopic/open common bile exploration was done, stent was removed intraoperatively after confirmation of no other stones in the biliary tract.

Identification and sensitivity testing were carried out by fully automatic VITEK 2 COMPACT system and susceptibility results were given in terms of MIC. The reports were generated after 72 hours.

Thorough clinical history was taken for exclusion and inclusion of patient for the study and to avoid any confounding factors. Patient was followed up for 1 month to see surgical site infection.

In this study we took it as that less than 72 hour's duration between ERCP stenting and cholecystectomy as early cholecystectomy and greater than 72 hours as late cholecystectomy.

To determine adult body mass index (BMI) by finding patient's height (in meters) and weight (in kg/m^2).

According to revised consensus body mass indices for Asian Indians: if BMI<18.5-underweight; BMI (18.5-22.9 kg/m^2) lean or normal weight; BMI (23-24.9 kg/m^2) overweight; BMI (≥25 kg/m^2) obese.

The International Expert Committee with members appointed by the ADA, the European Association for the study of Diabetes, and the International Diabetes Federation have issued diagnostic criteria for DM.

Criteria for the diagnosis of diabetes mellitus

Symptoms of diabetes+random blood sugar concentration ≥11.1 mmol/l (200 mg/dl) or fasting plasma glucose ≥7.0 mmol/l (126 mg/dl) or HbA1c ≥6.5% or 2-hour plasma glucose ≥11.1 mmol/l (200 mg/dl) during an oral glucose tolerance test.

For people without diabetes: normal range for the HbA1c level was between 4% and 5.6%.

For the people pre-diabetes: range for the HbA1c level was between 5.7% and 6.4%.

According to Tokyo Guidelines 2018, diagnostic criteria for acute cholangitis:

Systemic inflammation: A-1: fever (body temperature >38 ºC) and/or shaking chills; A-2: laboratory data: evidence of inflammatory response.

Cholestasis: B-1: jaundice (total bilirubin ≥2 mg/dl); B-2: laboratory data: abnormal liver function tests.

Imaging: C-1: biliary dilatation; C-2: evidence of the etiology on imaging (stricture, stone, stent)

Suspected diagnosis: one item in A+one item in either B or C.

Definite diagnosis: one item in A, one item in B and one item in C.

Note:

A-2: Abnormal white blood cell counts (<4 or >10×1,000 /µl), increase of serum C-reactive protein levels (≥1 mg/dl) and other changes indicating inflammation.

B-2: Increased serum ALP (>1.5×STDa IU), GGT (>1.5×STDa IU), AST (>1.5×STDa IU), and ALT (>1.5×STDa IU) levels.

Other factors which were helpful in diagnosis of acute cholangitis included abdominal pain (right upper quadrant or upper abdominal) and a history of biliary disease such as gallstones, previous biliary procedures, and placement of a biliary stent.

In acute hepatitis, marked systemic inflammatory response is observed infrequently. Virological and serological tests were required when differential diagnosis was difficult.

Criteria for defining a surgical site infection

Superficial incisional

Infection within 30 days after surgery involves skin and subcutaneous tissue only, plus one of the following purulent drainage; diagnosis of superficial surgical site infection by a surgeon; symptoms of erythema, pain, local edema.

Deep incisional

Infection within 30 days after surgery with soft tissue involvement. Infection within 1 year after surgery involves deep soft tissues (fascia and muscle), plus one of the following: purulent drainage from the deep space but no extension into the organ space; abscess found in the deep space on direct or radiologic examination or on reoperation; diagnosis of a deep space surgical site infection by the surgeon; symptoms of fever, pain and tenderness leading to wound dehiscence or opening by a surgeon.

Organ space

Infection within 30 days after surgery. Infection within 1 year after surgery with an infection; involves any part of the operation opened or manipulated, plus one of the following: purulent drainage from a drain placed in the organ space; cultured organisms from material aspirated from the organ space; abscess found on direct or radiologic examination or during reoperation; diagnosis of organ space infection by a surgeon.

Statistical analysis

Categorical variables were expressed as number of patients and percentage of patients and compared across the groups using Pearson's Chi square test for independence of attributes.

Continuous variables will be expressed as mean±standard deviation and compared across groups using unpaired t test/one way ANOVA if the data followed normal distribution and Mann-Whitney U test/Kruskal Wallis test if the data did not follow normal distribution.

The statistical software SPSS version 20 was used for the analysis. An alpha level of 5% would be considered, if any p value was less than 0.05 it was considered as significant.

Ethical considerations

There was no added risk involved in the study since only the data would be collected and analyzed of a procedure that has been scheduled as part of the patient's treatment protocol. There would be no additional cost involved in the study as bile culture and sensitivity was routinely

done after cholecystectomy in post endo biliary stented patients.

Ethical committee approval was obtained.

RESULTS

Statistical analysis

Statistical analysis was performed with help of Epi Info (TM) 7.2.2.2 EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC).

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviations (SD). Test of proportion was used to find the standard normal deviate (Z) to compare the difference proportions and Chi-square test was performed to find the associations. t test was used compare two means. $p<0.05$ was taken to be statistically significant.

Of the 50 post ERCP stented patients, who were undergone cholecystectomy either open or laparoscopically, we observed that 19 patients had diagnosed of cholelithiasis with choledocholithiasis, 10 patients had diagnosed of ACC with choledocholithiasis, 10 patients had diagnosed obstructive jaundice with cholecystocholedocholithiasis, 9 patients had diagnosed resolved gallstones pancreatitis with choledocholithiasis and remaining 2 cases were diagnosed with choledocholithiasis with chronic calculus cholecystitis. Diagnosis made after clinical examination with blood with radiological investigations.

Table 1: Distribution of post-operative complications of the study patients.

Post-operative complications	Number	%
Surgical site infection (SSI)	8	16.0
None	42	84.0
Total	50	100.0

Table 2: Distribution of sensitivity of different antibiotics to isolated bacteria from bile of studied patients.

Antibiotics	Isolated bacteria										Total (n=35)	
	E. faecium (n=1)		*E. gallinarum* (n=1)		*E. coli* (n=26)		*K. pneumoniae* (n=5)		*P. aeruginosa* (n=2)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Colistin	0	0.0	0	0.0	25	96.2	5	100.0	2	100.0	32	91.4
Tigecycline	1	100.0	1	100.0	26	100.0	4	80.0	0	0.0	32	91.4
Gentamicin	0	0.0	0	0.0	18	69.2	1	20.0	0	0.0	19	54.3
Amikacin	0	0.0	0	0.0	19	73.1	0	0.0	0	0.0	19	54.3
Cefepime	0	0.0	0	0.0	4	15.4	0	0.0	0	0.0	4	11.4
Ertapenem	0	0.0	0	0.0	4	15.4	0	0.0	0	0.0	4	11.4
Meropenem	0	0.0	0	0.0	4	15.4	0	0.0	0	0.0	4	11.4
Imipenem	0	0.0	0	0.0	4	15.4	0	0.0	0	0.0	4	11.4
Amoxicillin/ Clavulanic acid	0	0.0	0	0.0	3	11.5	0	0.0	0	0.0	3	8.6
Ampicillin	0	0.0	0	0.0	3	11.5	0	0.0	0	0.0	3	8.6
Cefaperazone/ Salbactum	0	0.0	0	0.0	3	11.5	0	0.0	0	0.0	3	8.6
Ceftriaxone	0	0.0	0	0.0	3	11.5	0	0.0	0	0.0	3	8.6
Ciprofloxacin	0	0.0	0	0.0	4	15.4	0	0.0	0	0.0	4	11.4
Teicoplanin	1	100.0	1	100.0	0	0.0	0	0.0	0	0.0	2	5.7
Sulphamethaxazole/ trimethoprim	0	0.0	0	0.0	3	11.5	1	20.0	0	0.0	4	11.4
Cefuroxime	0	0.0	0	0.0	3	11.5	0	0.0	0	0.0	3	8.6
Piperacillin/ Tazobactum	0	0.0	0	0.0	3	11.5	0	0.0	0	0.0	3	8.6
Erythromycin	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	2.8
Linezolid	1	100.0	1	100.0	0	0.0	0	0.0	0	0.0	2	5.7
Levofloxacin	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	2.8
Benzylpenicillin	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	2.8
Vancomycin	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.8
Tetracycline	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	2.8
Nil	1	100.0	0	0.0	8	30.8	2	50.0	0	0.0	11	31.4

Table 3: Distribution of resistance of different antibiotics to isolated bacteria from bile of studied patients.

Antibiotics	Isolated bacteria										Total (n=35)	
	E. faecium (n=1)		E. gallinarum (n=1)		E. coli (n=26)		K. pneumoniae (n=5)		P. aeruginosa (n=2)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ciprofloxacin	1	100.0	1	100.0	23	88.5	5	100.0	2	100.0	32	91.4
Amoxicillin+clavulanic acid	0	0.0	0	0.0	23	88.5	1	20.0	1	50.0	29	82.9
Cefaperazone+salbactum	0	0.0	0	0.0	23	88.5	5	100.0	1	50.0	29	82.9
Piperacillin+tazobactum	0	0.0	0	0.0	23	88.5	5	100.0	1	50.0	29	82.9
Ampicillin	0	0.0	0	0.0	23	88.5	5	100.0	0	0.0	28	80.0
Cefepime	0	0.0	0	0.0	22	84.6	5	100.0	1	50.0	28	80.0
Ceftriaxone	0	0.0	0	0.0	23	88.5	5	100.0	0	0.0	28	80.0
Cefuroxime	0	0.0	0	0.0	23	88.5	5	100.0	0	0.0	28	80.0
Imipenem	0	0.0	0	0.0	22	84.6	5	100.0	1	50.0	28	80.0
Meropenem	0	0.0	0	0.0	22	84.6	5	100.0	1	50.0	28	80.0
Trimethoprim+ sulfamethoxazole	0	0.0	0	0.0	24	88.5	5	100.0	1	50.0	30	85.7
Ertapenem	0	0.0	0	0.0	22	84.6	5	100.0	0	0.0	27	77.1
Gentamicin	1		1		7	26.9	2	40.0	1	50.0	32	91.4
Amikacin	0	0.0	0	0.0	7	26.9	4	80.0	1	50.0	12	34.3
Levofloxacin	1		0	0.0	0	0.0	0	0.0	1	50.0	2	5.7
Ceftazidime	0	0.0	0	0.0	0	0.0	0	0.0	1	50.0	1	2.9
Doripenem	0	0.0	0	0.0	0	0.0	0	0.0	1	50.0	1	2.9
Ticarcillin+clavulanic acid	0	0.0	0	0.0	0	0.0	0	0.0	1	50.0	1	2.9
Aztreonam	0	0.0	0	0.0	0	0.0	0	0.0	1	50.0	1	2.9
Benzylpenicillin	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.9
Erythromycin	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.9
Tetracycline	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.9
Nil	1	100.0	0	0.0	10	38.5	2	40.0	0	0.0	13	37.1

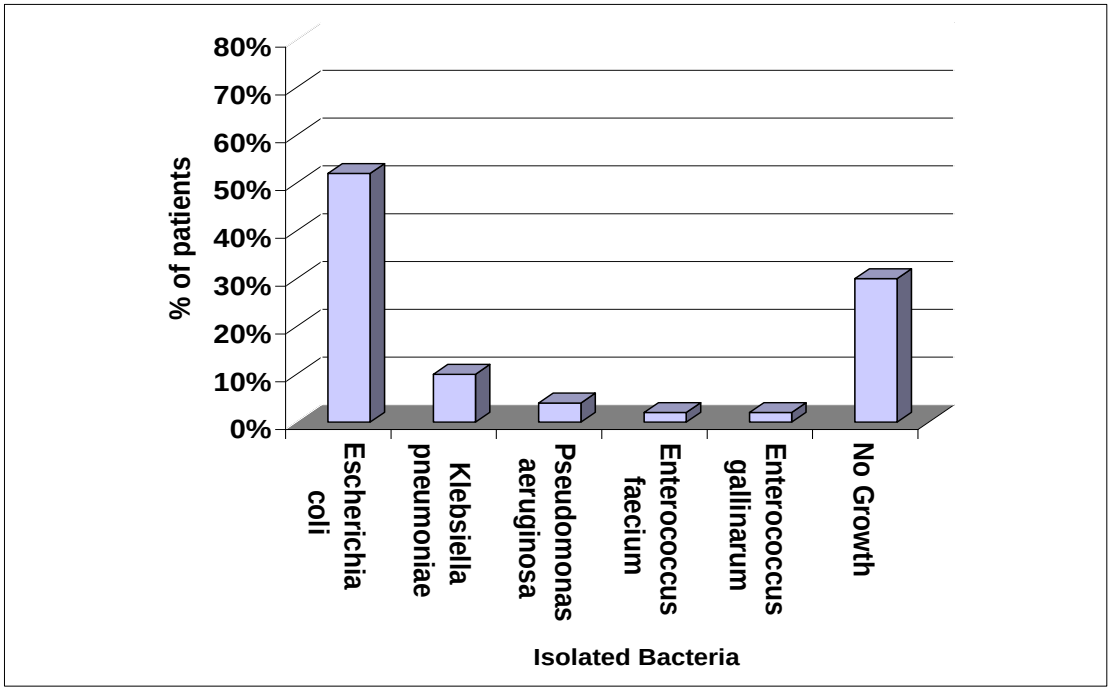

Figure 1: Distribution of Isolated Bacteria in positive bile culture of the study patients.

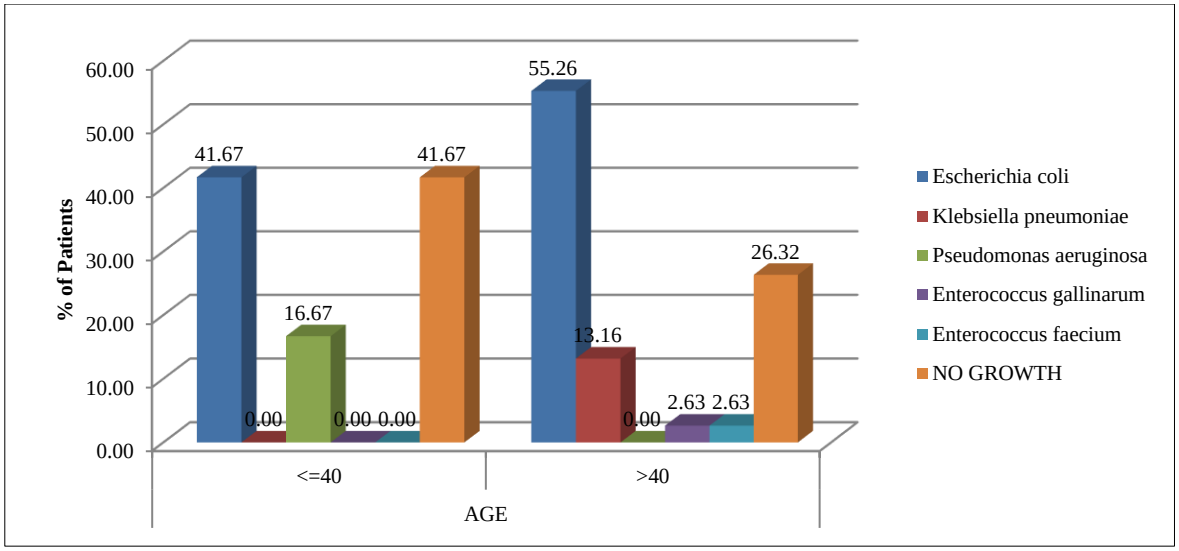

Figure 2: Distribution to show association between age and isolated bacteria from bile of study patients.

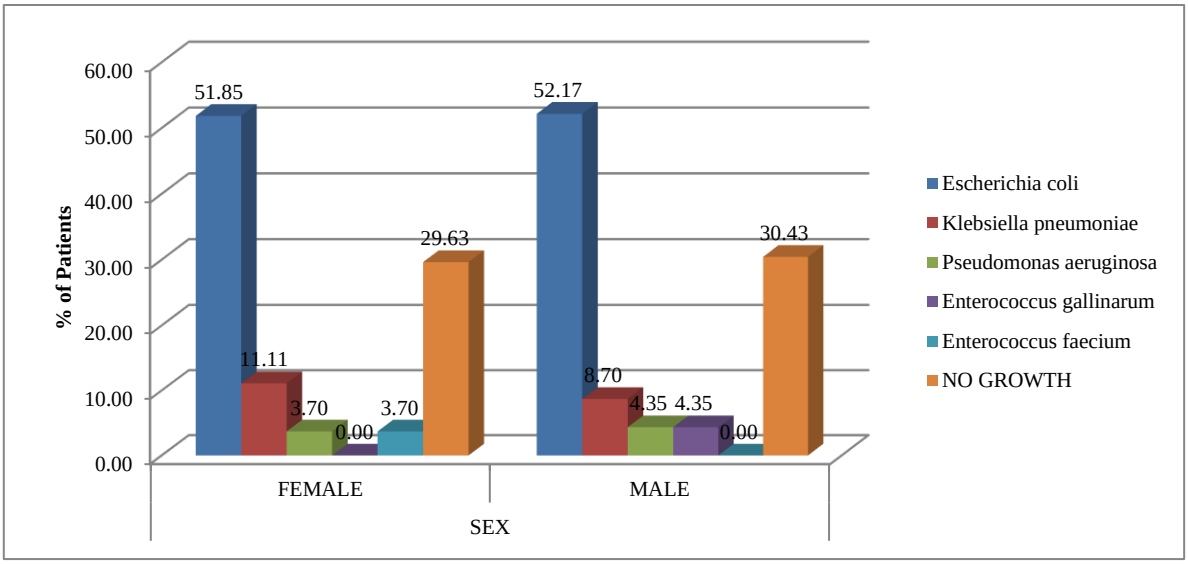

Figure 3: Distribution to show association between gender and isolated bacteria from bile of study patients.

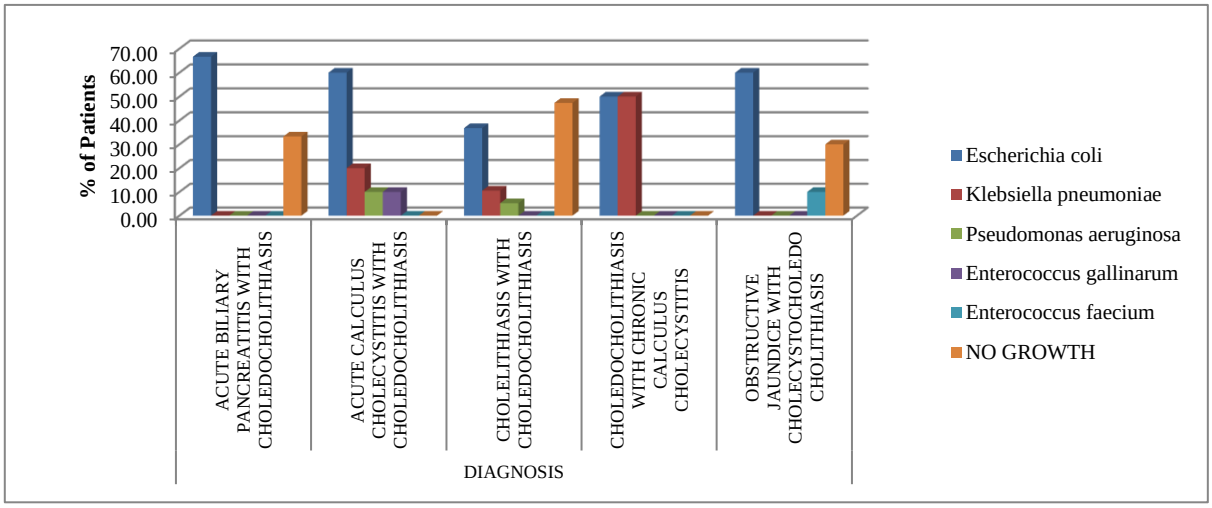

Figure 4: Distribution to show association between gender and isolated bacteria from bile of study patients.

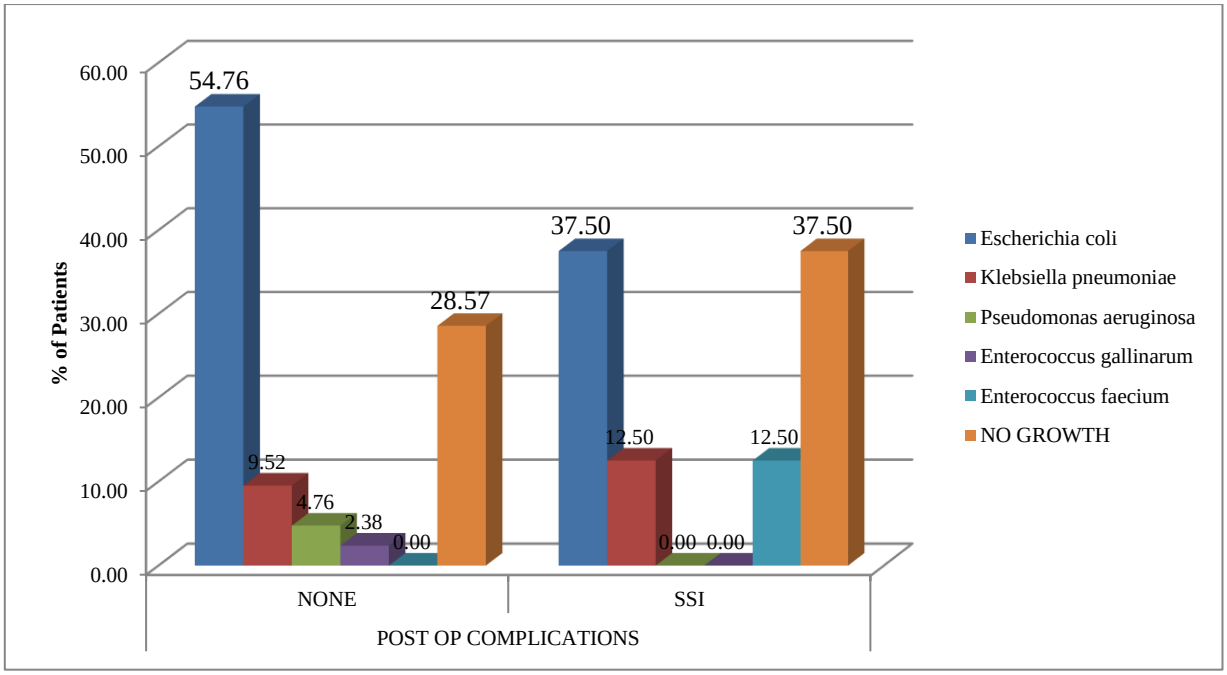

Figure 5: Distribution to show association between type of post-operative complications and isolated bacteria from bile of study patients.

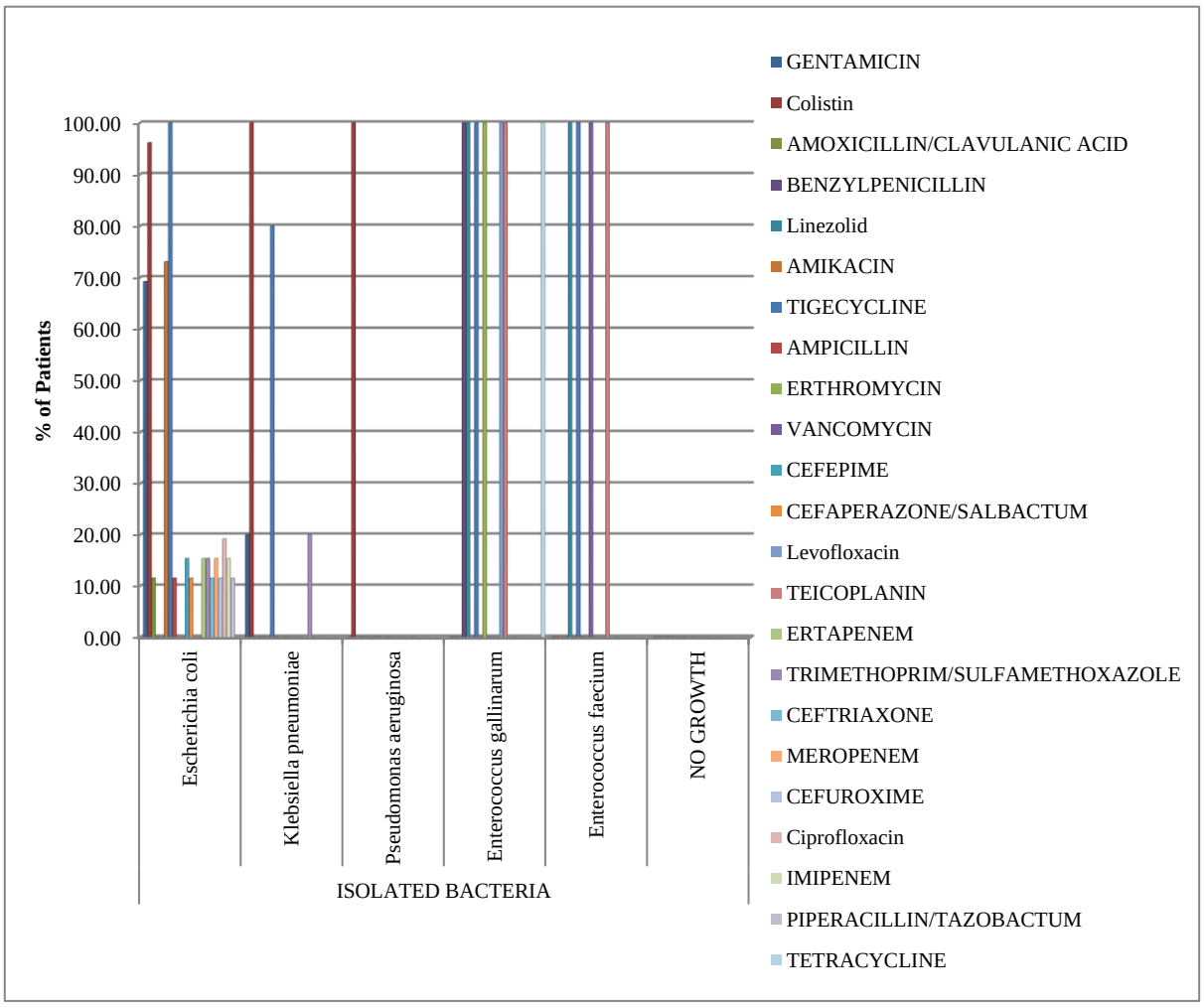

Figure 6: Distribution of sensitivity of different antibiotics to isolated bacteria from bile of studied patients.

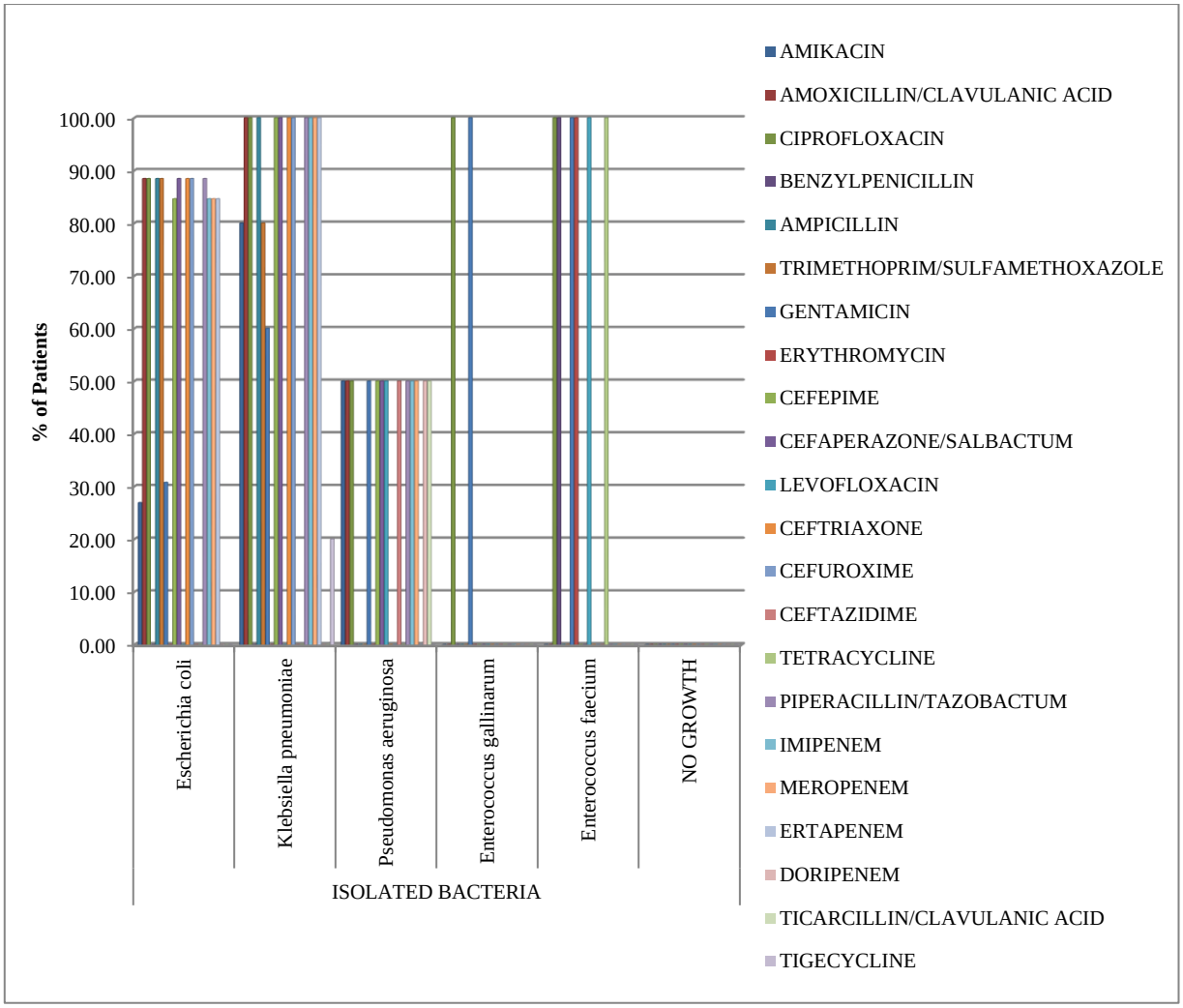

Figure 7: Distribution of resistance of different antibiotics to isolated bacteria from bile of studied patients.

Proportion of *E. coli* (52.0%) was the highest of all among the isolated bacteria which was significantly higher (Z=6.42: p<0.0001) (Figure1).

Only 8 (16.0%) of the patients had SSI but 84.0% of the patients had no post-operative complications which was significantly higher (Z= 9.61; p<0.0001) (Table 1).

There was no significant association between age and isolated bacteria (positive bile culture) (p value=0.109) (Figure 2).

There was no significant association between gender and isolated bacteria (positive bile culture) from bile of studied patients (p value=0.988) (Figure 3).

There was no statistically significant association between diagnosis and isolated bacteria from bile of studied patient (positive bile culture) (p value=0.114) (Figure 4).

There was no significant association between post-operative complication (SSI) and isolated bacteria from bile of the studied patients (p value=0.339) (Figure 5).

It was found out 2nd and 3rd generation cephalosporin (cefoperazone, cefepime, cefuroxime and ceftriaxone), piperacillin-tazobactum, imipenem, meropenem (higher group of antibiotics) showed high resistance (≥80%) among total isolated bacteria. Ciprofloxacin showed >90% resistance among total isolated bacteria. *K. pneumoniae* showed 100% resistance with *E. coli* showed >88% resistance against higher group of antibiotics (Table 2 and 3) (Figure 6 and 7).

DISCUSSION

Findings of this research work will guide use of most common antibiotic use in preoperatively, intraoperatively with postoperatively. We can also avoid postoperative complications and cost of treatment would be reduced. We also wanted to know what the incidence of SSI after

cholecystectomy in post stented patients and its association with the duration of hospital stay. This study also guided whether preoperative stenting was an independent risk factor for development for SSIs.

Of the 50 post stented ERCP patients, who were undergone cholecystectomy either open or laparoscopically, we observed that 19 patients had diagnosed of cholelithiasis with choledocholithiasis,10 patients had diagnosed of ACC with choledocholithiasis, 10 patients had diagnosed obstructive jaundice with cholecystocholedocholithiasis, 9 patients had diagnosed resolved gallstones pancreatitis with choledocholithiasis and remaining 2 cases were diagnosed with choledocholithiasis with chronic calculus cholecystitis. Most of the cases were cholelithiasis with choledocholithiasis (38.0%) which were significantly higher than other diagnosis ($Z=2.51$; $p<0.05$).

From my study, it was found that most of the studied patients are elder age groups, between 45-59 years (57.3%), which was higher ($Z=5.77$; $p<0.0001$). So, cholelithiasis with choledocholithiasis was more prevalent in elderly age groups.

There was female gender predominance (54%) as compared to male gender (46%) out of total 50 studied patients. But it was not significant ($Z=1.13$; $p>0.05$).

Females were in higher risk of having cholelithiasis with choledocholithiasis at a lower age than males. But there was no statistically significant association between age and gender of the patients ($p=0.08$).

Age and gender were independent risk factor for developing cholithiasis with choledocholithiasis. It was found to be there was no significant association between age and isolated bacteria from bile (positive bile culture) of the studied patients ($p=0.109$) and no significant association between gender and isolated bacteria (positive bile culture) of the studied patients ($p=0.988$).

Most of the patients had obesity (BMI $\geq$25 kg/m^2) (80.0%) which was significantly higher than the normal BMI ($Z=8.48$; $p<0.0001$). Thus, obesity was one of the significant risk factors of cholelithiasis with choledocholithiasis.

In my study, 30.0% of the patients had diabetes mellitus but 70.0% of the patients had non-diabetic, which was significantly higher ($Z=5.65$; $p<0.0001$). It was found that co-morbidities like diabetes mellitus type II was not significant risk factor for developing cholelithiasis with choledocholithiasis. In which 15 patients were found to have diabetes mellitus from total 50 patients. From my study it was shown that diabetes mellitus was significant association with isolated bacteria from bile from studied patients ($p=0.020$).

Of all patients undergone cholecystectomy in which 44 patients (88%) had undergone laparoscopic surgery and other 6 patients (12%) had open surgery due to intraoperative complications like Calot's anatomy seen difficulty/Calot's anatomy frozen with dense adhesions. Although in this duration in our institution a total number of more than 200 laparoscopic cholecystectomy was done in patients without prior ERCP stented patients, only 2 procedures were converted to open due to difficult hepato-biliary anatomy. Laparoscopic cholecystectomy was underwent which was significantly higher than laparoscopy converted to open cholecystectomy ($Z=10.74$; $p<0.001$). It was found out there was no significant association between type of surgical procedure done and positive bile culture of patients ($p=0.206$). There were 10 cases had intraoperative complications and 40 patients (80.0%) had no intra-operative complications which was significantly higher ($Z=8.48$; $p<0.0001$). It was found that there was no significant association between positive bile cultures with intraoperative complications ($p=0.086$).

In most of the cases mean time interval between ERCP and stenting and surgery of the patients was <5 days 72.0%) which was significantly higher ($Z=8.59$; $p<0.0001$).

In these 50 patients, bile culture and sensitivity samples were taken intraoperatively who had undergone ERCP followed by stenting, of this cultures, 35 patients (70%) had a positive bile culture and 15 patients (30%) had no growth in bile.

Of these 35 patients who had positive bile cultures, we found out that *E. coli* (74.29%) in 26 patients followed by *Klebsiella species* (14.3%) in 5 patients and *Pseudomonas species* (5.7%) in 2 patients were the most prevalent gram-negative bacteria isolates. As we found that over all, colistin (91.4%) and tigecycline (91.4%) followed by gentamicin (54.3%) and amikacin (54.3%) mostly were sensitive antibiotic. In *E. coli* was sensitive to colistin (100%) and tigecycline (96.2%), gentamicin (69.2%) and amikacin (73.1%). *Klebsiella sp.* was sensitive to colistin (100%), tigecycline (75%) and gentamicin (25%). Results also showed that certain strains of multi drug resistance *E. coli* in 6 patients were resistant to gentamicin/amikacin only sensitive to tigecycline and colistin, which was high. Other gram-positive bacteria, *Enterococcus Sp.* (4%) isolated in from bile culture and that was sensitive only to linezolid, levofloxacin and erythromycin.

Second and third generation cephalosporins, piperacillin-tazobactum (isolated Carbapenems >88%) were seen to be resistant about ≥80% in gram negative bacteria like *E. coli* and *Klebsiella pneumoniae* which were higher group of antibiotics as mentioned in results and analysis.

It was found that from results that SSIs are more prevalent in open cholecystectomy and found in those

patients who had bile culture positivity. But it may seem to be in those patients who had uncontrolled (high HbA1c) diabetes mellitus, elder patients had SSIs. Those patients who had undergone late (>72 hours) cholecystectomy had more chances of postoperative SSIs. It was found that there was no significant association between SSIs and bile culture positivity patients. The SSIs cause may be seem to multifactorial like age, co-morbidities like diabetes mellitus type II and gap or time interval between ERCP with stenting and cholecystectomy. As the ES may be the cause of bactibilia had more postoperative complications on delayed cholecystectomy.

In my study it was found that the time interval between ERCP with stenting and cholecystectomy had significant association of bile culture positivity (bactibilia). But the cause of this increase in bactibilia over time is still unknown. It suggests that destruction of the sphincter of oddi does in fact lead to reflux, resulting in increased colonization over time.[23] However, we also found that bactibilia in patients receiving ERCP followed by EC, making it plausible that bactibilia was present before sphincterotomy took place. This may suggest that GB or CBD stones themselves were a cause of colonization and ES added to this effect.[24]

Demographics factors like age, gender, BMI had not significant association with bile culture positivity and SSIs also. It may seem to be older age had more chances of bactibilia, but the cause may be unknown, may also related to immune system of individuals.[24]

Obesity with co-morbidities like uncontrolled diabetes mellitus type II may had bile culture positivity associated with postoperative complications like SSIs. But in our study, there was not significant association of bile culture positivity with postoperative complications like SSIs.

There was also not significant association of bile culture positivity with duration of hospital stay (dischargeable). As we found out from results those patients had postoperative SSIs and had OC had long duration of stay. But there was multifactorial reason for this hospital stay.

The limitations of this study were its low sample size (number of patients) and short duration of study. Also, we cannot do comparative study between post biliary stented and non-stented or uncomplicated patients.

CONCLUSION

In this study, we found that most prevalent isolates microorganism is gram negative bacteria that were mostly gut bacteria and the study indicates that the incidence of post ERCP infection is high and the drug resistance among the causative organism is common. Based on the results, there is scope of revisiting institutional prophylactic antibiotic policy. Thus, we suggest that the use of second or third generation cephalosporin or piperacillin-tazobactum as the antibiotic of choice for prophylaxis before ERCP may not be effective. Therefore, we recommend for administration of a prophylactic high grade antibiotic (colistin or tigecycline) before ERCP, which would target most biliary organisms and may also reduce the postoperative infectious complications. In addition, it was found that there was no significant association of a positive bile culture with risk of surgical site infections in patients undergoing biliary tract surgery.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Steiner CA, Bass EB, Talamini MA, Pitt HA, Steinberg EP. Surgical rates and operative mortality for open and laparoscopic cholecystectomy in Maryland. N Eng J Med. 1994;330(6);403-8.
2. Ko C, Lee S. Epidemiology and natural history of common bile duct stones and prediction of disease. Gastrointest Endosc. 2002;56(6):165.
3. Tierney S, Pitt HA. Choledocholithiasis and cholangitis. In: Bell RH, Rikkers LF, Mulholland MW, eds. Digestive tract surgery: a text and atlas. Philadelphia: Lippincott-Raven; 1996: 407-31.
4. Ahmed M, Kanotra R, Savani GT, Kotadiya F, Patel N, Tareen S, et al. Utilization trends in inpatient ERCP: A cross-sectional US experience. Endosc Int Open. 2017;5(4):261-71.
5. Harris A, Chan AC, Torres-Viera C, Hammett R, Carr-Locke D. Meta-analysis of antibiotic prophylaxis in endoscopic retrograde cholangio-pancreatography (ERCP). Endoscopy. 1999;31(9):126-30.
6. Reinders JS, Goud A, Timmer R, Kruyt PM, Witteman BJM, Smakman N, et al. Early laparoscopic cholecystectomy improves outcomes after endoscopic sphincterotomy for choledochocystolithiasis. Gastroenterology. 2010;138(7):2315-20.
7. Salman B, Yilmaz U, Kerem M, Bedirli A, Sare M, Sakrak O, et al. The timing of laparoscopic cholecystectomy after endoscopic retrograde cholangio-pancreatography in cholelithiasis coexisting with choledocolithiasis. J Hepatobiliary Pancreat Surg. 2009;16(6):832-6.
8. Sugiyama M, Atomi Y. Endoscopic Ultrasonography for diagnosing choledocholithiasis: A prospective comparative study with Ultrasonography and computed tomography. Gastrointest Endosc 1997;45(2):143-6.
9. Hallal AH, Jose D Amortegui, Jeroukhimov IM, Casillas J, Schulman CI, Manning RJ, et al. Magnetic resonance cholangio-pancreatography accurately detects common bile duct stones in

resolving gallstone pancreatitis. J Am Coll Surg. 2005;200(6):869-75.
10. Behrns KE, Ashley SW, Hunter JG, Carr-Locke D. Early ERCP for gallstone pancreatitis: for whom and when? J Gastrointest Surg. 2008;12(4):629-33.
11. Pokharel N, Rodrigues G, Shenoy G. Evaluation of septic complications in patients undergoing biliary surgery for gall stones in a tertiary care teaching hospital of south india. Kathmandu Univ Med J. 2007;5(3):371-3.
12. Deviere J, Motte S, Dumonceau JM, Serruys E, Thys JP, Cremer M. Septicemia after endoscopic retrograde cholangio-pancreatography. Endoscopy. 1990;22(2):72-5.
13. James EC, Collin DB. Sepsis complications in endoscopic retrograde cholangio-pancreatography. Am Surg. 1976;42(4):229-32.
14. Nomura T, Shirai Y, Hatakeyama K. Impact of bactibilia on the development of postoperative abdominal septic complications in patients with malignant biliary obstruction. Int Surg. 1999;84(3):204-8.
15. Mullen JT, Lee JH, Gomez HF, Ross WA, Fukami N, Wolff RA, et al. Pancreaticoduodenectomy after placement of endobiliary metal stents. J Gastrointest Surg. 2005;9(8):1094-105.
16. Jagannath P, Dhir V, Shrikhande S, Shah RC, Mullerpatan P, Mohandas KM. Effect of preoperative biliary stenting on immediate outcome after pancreaticoduodenectomy. Br J Surg. 2005;92(3):356-61.
17. Lissovoy G, Fraeman K, Hutchins V, Murphy D, Song D, Vaughn BB. Surgical site infection: Incidence and impact on hospital utilization and treatment costs. Am J Infect Control. 2009;37(5):387-97.
18. Dorman JP, Franklin ME, Glass JL. Laparoscopic common bile duct exploration by choledochotomy: an effective and efficient method of treatment of choledocholithiasis. Surgical Endoscopy. 1998;12(7):926-8.
19. Chandrakasan C, Reddy VV, Musunura BR, Reddy DA, Dasari V, Pagadala NNB. Correlation of intra-operative bile cultures with septic complications following biliary tract surgery. Int Surg J. 2016;3(4):2177-80.
20. Kondo K, Chijiiwa K, Ohuchida J. Selection of prophylactic antibiotics according to the microorganisms isolated from surgical site infections (SSIs) in a previous series of surgeries reduces SSI incidence after pancreaticoduodenectomy. J Hepatobiliary Pancreat Sci. 2013;20(3):286-93.
21. Khuroo MS, Mahajan R, Zargar SA, Javid G, Sapru S. Prevalance of biliary tract disease in India: a sonographic study in adult population in Kashmir. Gut. 1989;30(2):201-5.
22. Rai MK, Kumar V. Incidence of cholelithiasis in gallstone disease in eastern zone of India: a single centre study. Int J Med and Dent Sci. 2017;6(1):1417-20.
23. Mandryka Y, Klimczak J, Duszewski M, Kondras M, Modzelewski B. Bile duct infections as a late complication after endoscopic sphincterotomy. Pol Merkur Lekarski. 2006;21(126):525-7.
24. Maurer KJ, Carey MC, Fox JG. Roles of infection, inflammation, and the immune system in cholesterol gallstone formation. Gastroenterology. 2009;136(2):425-40.

Cite this article as: Dewangan KP, Geddam SR, Nayak PS. Microbiological profile of bile culture and its antimicrobial susceptibility pattern in endo-biliary stented patients undergoing cholecystectomy. Int Surg J 2022;9:1577-90.

APPENDIX I

Study proforma

Patient particulars:

Regn no.	Name	Age/sex
Address:		Contact No:
Date of Admission:		Consultant:

BMI: wt (in kg)/height^2 (in metre).

Clinical details:

Chief complains
Significant past medical history: diabetes mellitus type-ii (±)
Drug history

Clinical examination:

Investigations:

CBC: Hb:____gm/dl.
TLC:_____cell/cumm,
DLC:___%N,___%L,___%E,____%M,
PLT count: _____.
LFT: Bilirubin(T):_____(D):_____mg%, AST:_____U/L ALT:_____U/L ALK P:____U/L
RFT: Urea:_____mg/dL.
Creatinine:_____mg/dL,
Na/K:_____mEq/L
Blood Sugar:_____mg/Dl,
HbA_{1c}:_____%
Serology: HIV-1&2____,
HBsAg:_____, AntiHCV:_____.
ECG Findings:
Chest- X- ray:
ECHO:
USG:
ERCP:

Time interval between ERCP with stenting and surgery:

Diagnosis:

Surgical procedure:

Procedure: Standard Lap Cholecystectomy, Lap Cholecystectomy + CBDE, SILS Cholecystectomy, SILS Cholecystectomy + CBDE, Open Cholecystectomy, Open Cholecystectomy + CBDE.
Intraoperative Complication: Yes / No.
If yes, nature of complication:

Bile culture and sensitivity:

Organism Isolated	Sensitivity

Duration of hospital stay:

Post-operative any SSIs:

Date of discharge:

Withdrawal from the study: Yes/No If yes, cause: