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Original Research Article

Clinical Aspects of Bile Culture and Sensitivity in Cholelithiasis Patients Confirmed with Ultrasonography Undergoing Cholecystectomy in a Tertiary Care Hospital in Manipur.

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Abstract

Background: Usually the bile in the biliary system is sterile. However, the presence of gallstones within either the gallbladder or biliary tree is associated with bacterial colonization of the bile(1). Under conditions of normal bile flow, bacteria in the biliary system are of no clinical significance. Upon bile duct obstruction, eventually the bacteria presumably translocate into the circulation causing a systemic infection.

Aims: To evaluate the microbiological profile of bile from Cholelithiasis patients confirmed with Ultrasonography undergoing Cholecystectomy and to determine the antimicrobial sensitivity pattern.

Material and Methods: All patients for cholelithiasis confirmed with Ultrasonography Imaging in the Department of Radiodiagnosis are operated in the General Surgery Department between September,2019 to August,2021 in JNIMS, Imphal were included. Bile sample collected intra operatively in a sterile container and analyzed in Microbiology department.

Results: Total 170 patients participated in this study. Among these most common age group is 41- 50 years (47%),females are predominant (78.20%). n=37 among 170 patients showed growth in culture plate. Among the 37 patients who showed growth, 51-60 years age group showed maximum growth. Most common organism grown is E.coli (n – 16) (43.20%) followed by Klebsiella (n-11)(29.70%) and Pseudomonas aeroginosa (n-4)(10.8%). In this study the most overall sensitive antibiotic is Levofloxacin (75.7%) followed by Vancomycin (73%) , Imipenem(70.3%).Piperacillin & Tazobactam has the highest probability to be resistant(16.2%).

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Conclusion: The biliary tree inserts into the duodenum and therefore cannot be considered truly sterile. The most common types of bacteria found in biliary infections are Enterobacteriaceae, such as Escherichia coli, Klebsiella, and Enterobacter, followed by Enterococcus spp. Based on this study we would like to conclude that the Prophylactic antibiotics should be used in most patients undergoing interventions in the biliary tree, such as ERCP or PTC. To cover the most common bacterial species, a first- or second generation cephalosporin or fluoroquinolone should suffice.
Keywords: Cholelithiasis, Ultrasonography, Cholecystectomy, Bile, Enterobacteriaceae, Cephalosporin, Fluoroquinolone.

Introduction

Usually the bile in the biliary system is sterile. However, the presence of gallstones within either the gallbladder or biliary tree is associated with bacterial colonization of the bile.[1]In patients without stone disease, previous biliary intervention is associated with high rates of bacteriobilia.[2,3]Under conditions of normal bile flow, bacteria in the biliary system are of no clinical significance. Upon bile duct obstruction, bacteria proliferate within the stagnant bile while biliary pressure increases. Eventually, the bacteria presumably translocate into the circulation causing a systemic infection. Bacteriobilia is associated with higher postoperative infectious complications due to which there is prolonged hospital stay, need forantibiotics based therapy and higher cost.[4,5,6] Therefore, it is important to know the microbial flora of the gallbladder before prophylactic antibiotics are given.

Aims: This study aimed to evaluate the microbiological profile of bile from Cholelithiasis patientsconfirmed with Ultrasonography undergoing cholecystectomy and also to determine the antimicrobial sensitivity pattern.

Material and Methods: The study is prospective observational study, conducted in the Department of General Surgery, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur. Duration of the study was of two years , from September, 2019 to August, 2021. Patients aged between 18 years – 60 years undergoing laproscopic cholecystectomy, open cholecystectomy for cholelithiasis included in this study and with previous previous biliary interventions (eg, ERCP) within 1 week durations excluded from the study.

All patients with symptoms (pain over right hypochondrium, dyspepsia) were admitted from out-patient clinic of Surgical department for laparoscopic or open cholecystectomies after the diagnosis was confirmed on Ultrasonography scan in the Department of Radiodiagnosis JNIMS, Imphal (showing the gall bladder with calculi). Routine investigation like complete blood count; blood urea, serum creatinine, blood sugar; serum electrolytes and investigations for anesthesia fitness like chest X-ray, ECG and liver function tests were performed. An informed consent was obtained after explaining the purpose and procedure of the study. The patients were operated through open or laparoscopic cholecystectomy on the elective list by a single consultant surgeon. All patients were given an intravenous injection of antibiotic at induction of anesthesia and same antibiotics were given for 3 days postoperatively. After gaining access to the abdomen (laparoscopic or open), and recording the findings, bile was aspirated from gall bladder at the body of gall bladder in a 10 ml disposable syringe and kept

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in a sterile container provided from microbiology department for culture and sensitivity. Gall bladder was removed after ligation and cutting of the cystic artery and duct.

The collected specimen of the bile was labeled and sent to a single laboratory in a sterile container. Aerobic cultures of specimen were performed for microorganisms such as E. *coli, Klebseilla, Pseudomonas, Acinetobacter, Enterococcus and Staphylococcus* under the supervision of expert microbiologist. For aerobic culture, the sample was inoculated on blood agar and MacConkey agar medium and incubated at 37°C for 16 to 18 hours overnight. The culture plate was checked next day for bacterial growth. The colony morphology were studied and recorded.The colonies were subjected to various tests for identification of the isolates: i) Gram Stain ii) Hanging drop iii) Biochemical tests based on the Gram stain findings 1)Catalase test, 2) Coagulase test, 3) Oxidase test, 4) Citrate utilization test, 5) Urease production test, 6) Indole test, 7) Methyl red test, 8) VogesProskauer test, 9) Oxidative-fermentation (OF) test, 10) Nitrate reduction test, 11) Triple sugar iron test, 12) Sugar fermentation test.

Antibiotic susceptibility test: This was done by Kirby Bauer disk diffusion method on Mueller-Hinton agar according to Clinical Laboratory Standards Institute– CLSI (formerly NCCLS), 2018, M100 guidelines.

1) The Mueller-Hinton agar was prepared by reconstituting the readymade dehydrated powder as per the manufacturer's(HiMedia – Mumbai) guidelines.
2) 25ml of Mueller-Hinton agar medium was poured into petri-dishes of 9cm diameter, so that the depth of the medium is 4mm in thickness in accordance to CLSI guidelines.
3) The inoculum was prepared by picking three to five distinct colonies of approximately 1mm in diameter from a 24 hour old culture and suspending in 3-5ml of peptone water broth and incubating at 37°C for 4 to 6 hours.
4) The turbidity of the inoculum was adjusted to that of 0.5 Mac Farlandstandard.
5) A lawn culture of the inoculum was made on the Muller-Hinton agar using a sterile swab.
6) Antibiotic discs which were used are:-

A. For Gram Positive isolate: i) Azithromycin 15µg, ii) Trimethoprim – Sulphamethoxazole 1.25/23.75µg, iii) Linezolid 30µg, iv) Vancomycin 30µg, v) Tetracycline30µg, vi) Cefotaxime 30µg.
B. For Gram Negative isolate: i) Amoxicillin &Clavulanic acid 20/10µg, ii) Ceftriaxone30µg, iii) Levofloxacin 5µg, iv) Imipenem10µg, v) Gentamicin 10µg, vi) Amikacin 30µg.
C. For Non-Fermenters i) Piperacillin & Tazobactam 100/10µg, ii) Amikacin 30µg, iii) Levofloxacin 5µg, iv) Imipenem 10µg, v) Gentamicin 10µg, vi) Cefepime 30µg.
Zone of inhibition around the antibiotic disc in diameter was measured after incubating the plates for 24 hours at 37°C. The result was interpreted as sensitive (S), intermediate (I) and resistant (R)according to CLSI criteria M100.

Results: A total of 170 patients participated in this study. Among these most common age group is 41- 50 years (47%) as shown in Table 1,females are predominant (78.20%) as shown in Figure 1. n=37 among 170 patients showed growth in culture plate. Among the 37 patients who showed growth, 51-60 years age group showed maximum growth as shown in Table 2. Most common organism grown is E.coli (n – 16) (43.20%) followed by Klebsiella (n-11)(29.70%) and Pseudomonas aeroginosa (n-4)(10.8%) as shown in Figure 2. In this study the most overall sensitive antibiotic is Levofloxacin (75.7%) followed by Vancomycin (73%), Imipenem(70.3%).Piperacillin & Tazobactam has the highest probability to be resistant(16.2%) as shown in Table 3.

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Table 1: Descriptive analysis of age group in study population (N=170)

Age group	Frequency	Percentage
<30	5	2.9%
31-40	38	22.4%
41-50	81	47.6%
51-60	37	21.8%
>60	9	5.3%
Total	170	100.0%

Among these most common age group is 41- 50 years (47%)

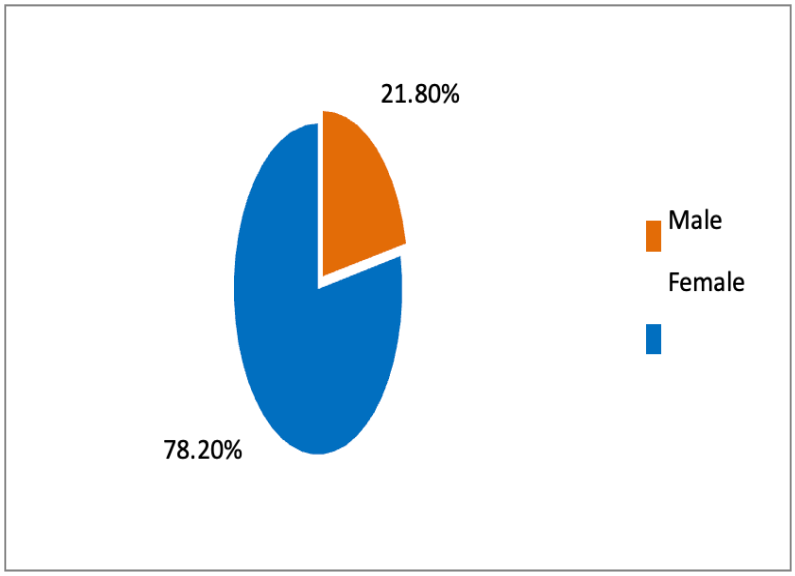

Figure 1: Pie chart of gender

Only n=37 among the 170 patients showed growth in culture plate

Table 2: Comparison of age group with gender (N=37)

Age group	Gender		Total
	Male	Female	
31-40	0	1	1
41-50	6	8	14
51-60	2	13	15
>60	1	6	7
Total	9	28	37

Among the 37 patients who showed growth, 51-60 years age group showed maximum number of study population

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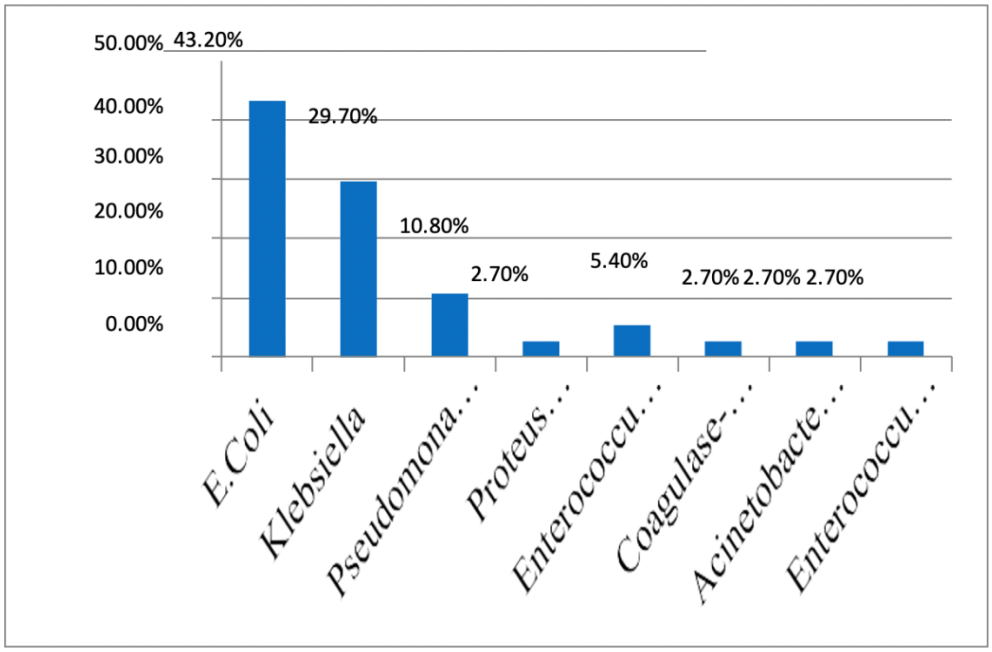

Figure 2: Bar chart of organism

Most common organism grown is E.coli (n – 16)(43.20%)followed by Klebsiella (n-11) (29.70%) and Pseudomonas aeroginosa (n-4) (10.8%).

Table 3: Descriptive analysis of culture sensitivity in study population (N=37)

	Sensitive	Resistant	Intermediate Sensitive
Azithromycin	20 (54.1%)	15 (40.5%)	2 (5.4%)
Trimethoprim – Sulphamethoxazole	21 (56.8%)	16 (43.2%)	-
Linezolid	24 (64.9%)	10 (27%)	3 (8.1%)
Vancomycin	27 (73%)	6 (16.2%)	4 (10.8%)
Tetracycline	25 (67.6%)	12 (32.4%)	-
Cefotaxime	23 (62.2%)	14 (37.8%)	-
Amoxicillin &Clavulanic acid	21 (56.8%)	16 (43.2%)	-
Ceftriaxone	22 (59.5%)	14 (37.8%)	1 (2.7%)
Levofloxacin	28 (75.7%)	8 (21.6%)	1 (2.7%)
Imipenem	26 (70.3%)	10 (27%)	1 (2.7%)
Gentamicin	25 (67.6%)	12 (32.4%)	-
Amikacin	23 (62.2%)	11 (29.7%)	3 (8.1%)
Piperacillin & Tazobactam	23 (62.2%)	8 (21.6%)	6 (16.2%)

In this study highest sensitivity for overall is observed for levofloxacin (75.7%) followed by vancomycin (73%), imipemam(70.3%) and piperacillin &Tazobactam has the lowest

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Table 4: Descriptive analysis of organism and Culture Sensitivity (N=37)

Culture Sensitive	Organism							
	E.Coli	Klebsiella	Pseudomonas aeruginosa	Proteus mirabilis	Enterococcus–faecium	Coagulase-negative	Acinetobacter baumani	Enterococcus species
Azithromycin	9 (45%)	5 (25%)	2 (10%)	-	2 (10%)	1 (5%)	1 (5%)	-
Trimethoprim–Sulphamethoxazole	10 (47.6%)	4 (19%)	3 (14.3%)	-	2 (9.5%)	1 (4.8%)	1 (4.8%)	-
Linezolid	11 (45.8%)	5 (20.8%)	3 (12.5%)	1 (4.2%)	2 (8.3%)	1 (4.2%)	1 (4.2%)	-
Vancomycin	13 (48.1%)	6 (22.2%	3 (11.1%)	1 (3.7%)	2 (7.4%)	1 (3.7%)	1 (3.7%)	-
Tetracycline	10 (40%)	7 (28%)	3 (12%)	1 (4%)	2 (8%)	1 (4%)	1 (4%)	-
Cefotaxime	8 (34.8%)	8 (34.8%)	2 (8.7%)	1 (4.3%)	2 (8.7%)	1 (4.3%)	1 (4.3%)	-
Amoxicillin &Clavulanic acid	7 (33.3%)	6 (28.6%)	2 (9.5%)	1 (4.8%)	2 (9.5%)	1 (4.8%)	1 (4.8%)	1 (4.8%)
Ceftriaxone	10 (45.5%)	4 (18.2%)	2 (9.1%)	1 (4.5%)	2 (9.1%)	1 (4.5%)	1 (4.5%)	1 (4.5%)
Levofloxacin	10 (35.7%)	8 (28.6%)	4 (14.3%)	1 (3.6%)	2 (7.1%)	1 (3.6%)	1 (3.6%)	1 (3.6%)
Imipenem	10 (38.5%)	7 (26.9%)	3 (11.5%)	1 (3.8%)	2 (7.7%)	1 (3.8%)	1 (3.8%)	1 (3.8%)
Gentamicin	11 (44%)	6 (24%)	2 (8%)	1 (4%)	2 (8%)	1 (4%)	1 (4%)	1 (4%)
Amikacin	10 (43.5%)	6 (26.1%)	1 (4.3%)	1 (4.3%)	2 (8.7%)	1 (4.3%)	1 (4.3%)	1 (4.3%)
Piperacillin&Tazobactam	9 (39.1%)	7 (30.4%)	2 (8.4%)	1 (4.3%)	2 (8.4%)	-	1 (4.3%)	1 (4.3%)

highest sensitivity forVancomycin (48.1%) followed by Klebsiella towards cefotaxime (34.8%) and pseudomonas for Levoloxacin(14.3%) as shown in Table 4.

Discussion:

Gallstone disease is one of the most common problems affecting the digestive tract. Autopsy reports have shown prevalence of 11% to 36%.[12] Prevalence of gallstone ranges from 10 to 20% in India.[13] It affects nearly 4.3% of the Indian population.[14] In India it is seven times more common in north than southern parts of the country[15]. Bile in the gallbladder or bile ducts in the absence of gallstones or any other biliary tract disease is normally sterile. In the presence of gallstones or biliary obstruction, theprevalence of bactibilia increases.

In the present study 170 bile samples were analyzed for the presence ofmicroorganism out of which 37(21.8%) showed bacterial growth which was in accordance with *Ahmed M et al*(23.6%)[7]while studies conducted by *Hadi YB et al* [8]and *Capoor MR et al*[9]found higher rate of bacterial growth i.e.(33.6%) and (32%) respectively.The demographic data of this study was found to be M:F = 1:3.5 ,males 21.8% and females 78.2%.The age pattern for patients presenting for cholecystectomy showed that most of the patients (81;47.6%) belonged to the 4thand 5th decades of life.

The results of this study is comparable with the study by *Ahmaad Maqsood et al.*[10] In their study 2015,106 patients with cholecystectomy for symptomatic gallstones showed positive bile culture in only 25 patients (23.6%). *E. coli* was the most common cultured organism in 10 (40%) patients, *Klebsiella* in five (20%) patients, *Pseudomonas* in five (20%) patients, *Proteus*

in two (8%) patients, *Staphlococcus aureus* in two (8%) patients, and mixedorganisms were cultured in one patient (4%). Cefoperazone with sulbactum and amikacin were the most effective antibiotics.

In a study by *Christian Rupp et al.*[11]in 2016, *Enterococcus species* was predominant (494/1150) followed by *E. coli and Klebsillaspp*(179/1150). Gram Negative Bacteria were sensitive to 2nd generation cephalosporins, carbapenems. The combination of carbapenems and vancomycin was effective in seriously ill patients. Multidrug resistance strains were found in 11.3% of all samples.

In a study by *Ozturk et al.*[16] in 2012,114 patients who underwent cholecystectomy for various reasons showed bacterial growth in the bile culture in 15 patients (13.1%). The most commonly isolated bacteria were Enterococcus spp (four patients, 26.6%), Escherichia coli (three patients, 20%) and Enterobacter spp (three patients, 20%). The bile culture positivity rate was highest in patients with acute cholecystitis combined with choledocolithiasis (three patients, 100%). The bile culture bacterial growth was highest in patients over 60 years of age (10 patients, 27 %) and in those with concomitant illness (nine patients, 23.6%).

In a study by Malini R. Capoor[17] in 2008, the median age for the patients was 41yrs, male female ratio is 0.48. thirteen out of 134 samples were culture positive. Most common organism isolated was *E. coli* (29.7%), *Klebsiellapneumonae* (27%), *Salmonella entericaserovartyphi* (8.1%), *Pseudomonas aeruginosa* (5.4%). The majority of Enterobacteriaceae isolates were susceptible to piperacillin –tazobactum and meropenem. Multidrug resistant GI organisms such as *P. aeruginosa*, *Acinetobacter* spp., *S. aureus* were more frequently observed in patients with acute cholecystitis with gastrointestinal aliments

In the study by Suri *et al.*[18]in study 2009, the highest incidence of positive cultures was noted in patients with acute cholecystitis (40%) although this difference was statistically not significant due to P value of 0.1266.

Van Leeuren PA *et al.*[19] in a 1985 study, out of a total 840 patients ,138 patientsshowed positive bile culture (16.4%) and 72 patients showed wound infection (9%). Bile culture showed E. coli was most common organism isolated (36%). But there was no correlation between positive bile culture and subsequent wound culture.

Valazquez-Mendoza JD et al.[20]in 2010 study; total 80 patients study, 40 patientswith bile culture positive and 40 patients with wound culture positive. There was no statistically significant difference when comparing surgical site infection in both groups.

Conclusion:

The biliary tree inserts into the duodenum and therefore cannot be considered truly sterile. Through a low bacterial load and with the flow of bile, infection in the absence of obstruction is rare. However, with the presence of stones or obstruction, the likelihood of bacterial infection increases. The most common types of bacteria found in biliary infections are Enterobacteriaceae, such as *Escherichia coli*, *Klebsiella*, and E*nterobacter*, followed by *Enterococcus* spp. Based on this study we would like to conclude that Prophylactic antibiotics should be used in most patients undergoing interventions in the biliary tree, suchas ERCP or PTC. To cover the most common bacterial species, a first- or second generation cephalosporin or fluoroquinolone should suffice.

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References:

1. Csendes A, Fernandez M, Uribe P. Bacteriology of the gallbladder bile in normal subjects. *Am J Surg*1975; 129: 629-631.
2. *Hochwald SN*, Burke EC, Jarnagin WR, Fong Y, Blumgart LH. Association ofpreoperative biliary stenting with increased postoperative infectious complications in proximal cholangiocarcinoma. *Arch Surg* 1999; 134: 261-266.
3. Nomura T, Shirai Y, Hatakeyama K. Bacteribilia and cholangitis after percutaneous transhepatic biliary drainage for malignant biliary obstruction. *Dig Dis Sci* 1999; 44: 542-546
4. Lai EC, Mok FP, Tan ES, *et al.,* Endoscopic biliary drainage for severe acute cholangitis. *N Engl J Med* 1992; 326: 1582–1586.
5. Lax AJ, Thomas W. How bacteria could cause cancer: one step at a time. Trends in Microbiol. 2002; 10: 293-9.
6. Lee DW, Chung SC. Biliary infection. Bailliere ClinGastroenterol. 1997; 11: 707–24.
7. Ahmed M, Akhtar MR, Ali A et al. Microbiology of bile in symptomatic uncomplicated gallstone diseases. Pak Armed Forces Med J. 2015; 65(4): 491-93.
8. Hadi YB, Waqas M, Umer HM et al. Bacterobilia in acute cholecystitis: bile cultures' Isolates. JPMA: Journal of Pakistan Medical Association. 2016; 66(10):50-52
9. Capoor MR, Nair D, Khanna G et al. Microflora of Bile Aspirates in Patients with Acute Cholecystitis With or Without Cholelithiasis: A Tropical Experience. TheBrazilian Journal of Infectious Diseases. 2008; 12(3):222-225
10. Ahmad M, Akhtar MR, Ali A, Ahmad A, Hashmi JS. Microbiology of bile in symptomatic uncomplicated gallstone Disease. Pak Armed Forces Med J. 2015;65(4):491-3
11. Rupp C, Bode K, Weiss KH, *et al.,* Microbiological assessment of bile and corresponding antibiotic treatment. Medicine 2016 vol. 95(10).
12. Brunicardi FC. Gallbladder and the extrahepatic biliary system. In: Anderson DK, Billiar TR, Dunn DL, Hunter JG, Matthews JB, Pollock RE. Schwart's Principles of Surgery. 10th ed. New York : McGraw Hill; 2015.p. 1309-40.
13. Sherry CK. Open Cholecystectomy. Am J Surg 1993;165:435-9.
14. Choy I, Kitto S, Adu-Aryee N, Okrainec A. Barriers to the uptake of laparoscopic surgery in a lowermiddle-income country. Surgical Endoscopy 2013;27(11):4009-15.
15. Mohan H, Punia RP, Dhawan SB, Ahal S, Sekhon MS. Morphological spectrum of gallstone disease in 1100 cholecystectomies in North India. Indian J Surg 2005;67(3):22-29.
16. Ozturk A, Bozkutoglu H, Kaya C, Tan N, Çaskurlu H, Akinci UF. Bacteriologic analysis of bile in cholecystectomy patients. N J Med. 2012;29:43-6
17. Capoor M, Nair D, Khanna G, *et al.,* Microflora of bile aspirates in patients with acute cholecystitis with or without cholelithiasis: a tropical experience. The Brazilian journal of infectious disease 2008; 12(3): 222-225
18. Suri A, Yasir M, Kapoor M, Aiman A, Kumar A. Prospective study on biliarybacteriology in Calcular disease of the gall bladder and the role of common newer antibiotics. Internet J Surg. 2009;22(2):1.
19. Van Leeuwen PA, Keeman JN, Butzelaar RM, van den Bogaard AE. Correlation between a positive gallbladder culture and subsequent wound infection after biliary surgery - a retrospective study of 840 patients. Neth J Surg. 1985;37:179-82.
20. Valazquez-Mendoza JD, Alvarez-Mora M, Velázquez-Morales CA, Anaya-Prado R. Bactibilia and surgical site infection after open cholecystectomy. Cir Cir. 2010;78(3):239-43