



ORIGINAL RESEARCH PAPER

General Surgery

STUDY OF FREQUENCY AND SPECTRUM OF MICROBIAL GROWTH AND ANTIBIOTIC SENSITIVITY PATTERN IN BILE CULTURE IN PATIENTS UNDERGOING CHOLECYSTECTOMY - A HOSPITAL BASED STUDY

KEY WORDS: Gall bladder. Bile.Cholecystitis. Sensitivity. Cholecystectomy. Bacteria.Culture

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ABSTRACT

This study is carried out to determine the current trend of bacteriology and their sensitivity to common antibiotics in our population with symptomatic cholelithiasis. Total number of patient undergone cholecystectomy with symptomatic cholelithiasis were 110. Out of these male and female patients were 23(20.91%) and 87 (79.1%) respectively with male to female ratio of 1 : 3.78. The mean age of male and female patients with symptomatic cholecystitis were 46.20±10.88 years and 45.95±10.14 years. On culture and sensitivity test, 52(47.28%) have positive growth while 58 (52.73%) has no growth. The most common bacteria isolated was E Coli 28(25.45%) followed by klebsiella 16(14.54%), Salmonella 3(2.73%) and Shigella 2(1.81%), Pseudomonas 1(0.90%) & Acinetobactor 2(1.81%). Maximum no of patients with symptomatic cholelithiasis were 45(40.91%) that belong to the age group of 41 to 50 years followed by 38(34.54%) from the age group of 31 to 40 years. As per age wise distribution of isolated bacteria in symptomatic cholecystitis on culture test of bile, E Coli was most common in age group of 31 to 40 years; 15(13.63%), Klebsiella was common in age group of 41 to 50 years; 8(7.27%). According to gender wise distribution of isolated bacteria in symptomatic cholelithiasis on culture sensitivity, E Coli was isolated in 8(7.26%) males and 20 (18.18%) females, Klebsiella in 5(5.55%) males and 11(10%) females. 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 shows high resistance to Ampicillin in 17(60.71%) patients followed by resistance to Gentamycine in 12(42.86%) patients. Klebsiella showed high sensitivity to Gentamycine in 13(81.25%), Cefuroxime 12(75%), patients. The resistance of Klebsiella was noted maximum to Ampicillin which was in 10(62.5%) patients followed by resistance to Moxifloxacin in 8(50%). Salmonella showed high sensitivity to Cefuroxime in 3(100%) while the resistance was high to Ampicillin 3(100%) patients. Shigella showed high sensitivity to Gentamycine in 2(100%) and Cefuroxime 2(100%) cases. The resistance of Shigella was noted in maximum to Ampicillin in 2(100%) patients. The only culture grown pseudomonas was sensitive to Cefuroxime & Gentamycine and resistant to Moxifloxacin and Ampicillin. The sensitivity of acinetobactor was high in Cefuroxime 2(100%), Gentamycine 2(100%) & Ceftriaxone 2(100%), both the Acinetobactor growth were resistant to Ampicillin & Moxifloxacin.

Introduction:

Acute cholecystitis is an infection of biliary tract, which results from bile stasis due to chronic obstruction. The obstruction is usually attributed to gallstones in 80% of cases. Gall stones can be divided in to three main types : Cholesterol, pigment (brown/black) or mixed stones. The causes of acalculus cholecystitis include biliary strictures, human immunodeficiency virus cholangiopathy, biliary parasites and primary sclerosing cholangitis. Other causes include complicated cases of burns, trauma, major surgery, Diabetes and unusual bacterial infection of gall bladder (Salmonella spp, or Vibrio cholerae) and other systemic infections (Tuberculosis or syphilis). Human bile though sterile normally can become infected due to obstruction. Obstruction causes an increase in ductal pressure which leads to entry of micro organism through various routes like papilla of Vater or hematogenous resulting in bacterial proliferation and dissemination. Bacterial cholangitis is the most common type of acute cholangitis, with a gram negative preponderance. Gram positive and anaerobic are uncommon causative agents. Viral and fungal agents are rare.

Although this disease has a low mortality rate, its economic and health impact is significant due to its high morbidity. In fact gallstone disease is one of the most common abdominal condition both in developed and developing countries for which patients are admitted to hospitals and its frequency is increasing. Approximately 1-2% of asymptomatic patients will develop symptom requiring

Cholecystectomy per year, making cholecystectomy one of the most common operations performed by general surgeons. Therefore there is the need for more knowledge of the epidemiological characteristics of GD in order to better identify therapeutic strategies.

The pathogenesis of bile infection is incompletely understood, with prevailing theories not fully explaining all the observations. There is relatively sparse data, both local and international on the

prevalence of the infection in patients undergoing cholecystectomy. The conservative and prophylactic treatment is therefore based on best guess basis. The changing sensitivity pattern of antibiotics also pose a therapeutic challenge to clinicians.

The rationale of this study was to determine the current trend of bacteriology and their sensitivity to common antibiotics in our population with symptomatic cholelithiasis. The results of this study will be used to develop guidelines and recommendations for the rationale use of antibiotics. The result of this study will be shared with all surgeons and general practitioners locally and in the periphery to help them identify the type of antibiotic to be administered in symptomatic cholelithiasis. This will help us in rational use of antibiotic as well as reducing morbidity associated with cholelithiasis.

Material and Method:

Patients with cholelithiasis, undergone cholecystectomy in AGMC & GBPH hospital who are more than 18 years old, irrespective of sex, and religion is included in this study.

STUDY DESIGN: Descriptive cross sectional study **STUDY SETUP:** The study was carried out In the Department of Surgery AGMC & GBPH, Agartala. Tripura. **STUDY DURATION:** The procedure and data collection carried out for one year, with effect from the month of 01st January 2013 to 31st December 2013

STUDY POPULATION: **SAMPLE SIZE:** In department of Surgery AGMC and GBPH from Aug 2011 to Sep 2012 a total no of 1430 major operations has been done. Out of that 480 were complicated or uncomplicated cholecystectomies. Prevalence of cholecystectomy 34%. $q = (100 - 34) = 66$

$L = 20\%$ of prevalence = 13
 $n = (1.96)^2 \times 34 \times 66 / (13)^2$
 So the sample size (n) = 53

Though the sample size was 53, however all the patients older than 18 years undergoing cholecystectomy from 01st 2013 to 31st December in AGMC & GBPH, Department of Surgery is included in the study and a total no of 126 patients were studied during the period.

Patients of cholelithiasis, older than 18 years of age who has undergone cholecystectomy in AGMC & GBPH are included in this study.

Patients not undergoing, unwilling or unfit for surgery. Patients with obstructive jaundice (raised Alkaline phosphatase more than two times of normal) METHOD :All the study patients presenting with symptoms (pain right hypochondrium and vomiting) and tender right hypochondrium was diagnosed clinically as Cholecystitis and the diagnosis was confirmed by Ultrasonography (showing distended gall bladder with calculi).Routine investigations like Complete blood count , Blood Urea & Serum Creatinine, Random blood Sugar, Serum electrolytes and investigations for anesthesia fitness like Chest X-Ray, ECG and LFT were performed. Pre anaesthetic checkup was done and clearance was explained to the patients . Informed consent is taken from each patient. The aims and objectives of the study, the benefits to be obtained, the confidentiality of participants and results, the voluntary nature of participation and free-will to withdraw from the study without penalty were clearly spelt out to the participants.

All the patients were operated through open cholecystectomy on subsequent elective list by consultant Surgeons. All patients were given a IV injection of third generation Cephalosporins at induction of anaesthesia and was continued for 3 days post operatively. After opening the abdomen and recording the findings, bile was aspirated from gall bladder at fundus in a 5ml disposable syringe. Gall bladder was removed after ligation and cutting of the cystic artery and duct.

TRANSPORT OF SAMPLE : The collected specimen of bile was labeled and send to Microbiology lab, AGMC & GBPH in 5cc disposable syringe.

CULTURE & SENSITIVITY METHOD : The macroscopic appearance of the sample was noted. Direct identification of organism by gram staining was done. For aerobic culture, the sample was inoculated on blood agar medium and incubated at 37° C for 24 hours.Isolation of the isolates was done by standard biochemical tests.Antibiogram of isolated organism is prepared by Kirby bauer method following CLSI guidelines.

ANALYSIS OF DATA : Data was recorded in a structured proforma, entered and analyzed using Microsoft MS Excel move. Patients were distributed according to age and gender. Percentage of different bacterial growth & sensitivity pattern on antibiogram was tabulated according to age and sex. The data was presented in the form of table and charts.

Results: Total number of patient undergone cholecystectomy with symptomatic cholelithiasis were 110. Out of these male and female patients were 23(20.91%) and 87 (79.1%) respectively with male to female ratio of 1 : 3.78.

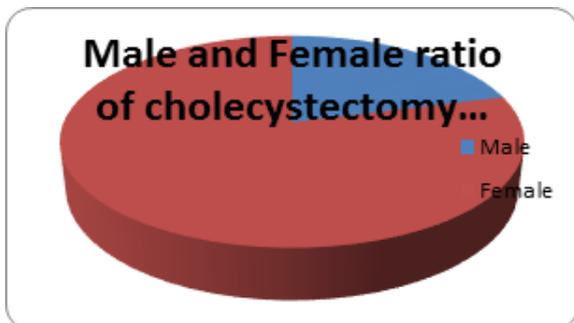


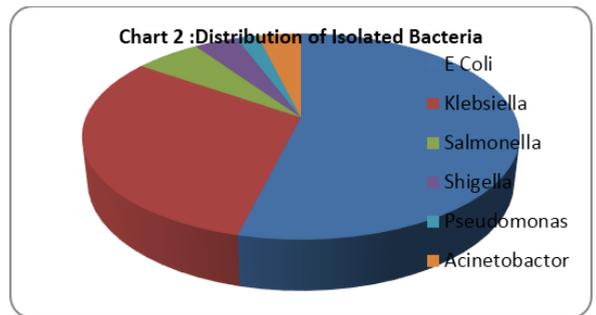
Chart 1 : Male & Female ratio of Cholecystectomy patients.

The mean age of male and female patients with symptomatic cholecystitis were 46.20±10.88 years and 45.95±10.14 years respectively with an overall mean age of 46.13±10.65 years (table 1).

Table 1 : Mean age ± standard deviation of patients with symptomatic cholecystitis

Gender	Mean age ± standard deviation(SD)
Male	46.20±10.88
Female	45.95±10.14
Total	46.13±10.65

On culture and sensitivity test, 52(47.28%) have positive growthwhile 58 (52.73%) has no growth.The most common bacteria isolated was E Coli 28(25.45%) followed by klebsiella 16(14.54%), Salmonella 3(2.73%) and Shigella 2(1.81%), Pseudomonas 1(0.90%) & Acinetobactor 2(1.81%).



Maximum no of patients with symptomatic cholelithiasis were 45(40.91%) that belong to the age group of 41 to 50 years followed by 38(34.54%) from the age group of 31 to 40 years. As per age wise distribution of isolated bacteria in symptomatic cholecystitis on culture test of bile, E Coli was most common in age group of 31 to 40 years; 15(13.63%), Klebsiella was common in age group of 41 to 50 years; 8(7.27%). Full detail of age wise distribution is shown in table 2.

Table 2: Age wise distribution of common bacterial isolates on culture and sensitivity of bile in patients with symptomatic cholelithiasis.

Age groups (years)	E Coli n=28(25.45%)	Klebsiella n=16(14.54%)	Salmonella n=3(2.73%)	Shigella n=2(1.81%)	Pseudomonas n=1(0.90%)	Acinetobactor. n=2(1.81%)
18-30	2 (1.81%)	1 (0.90%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
31-40	10 (9.09%)	4 (3.64%)	1 (0.90%)	0 (0%)	0 (0%)	2 (1.81%)
41-50	15 (13.63%)	8 (7.27%)	2 (1.81%)	2 (1.81%)	0 (0%)	0 (0%)
51-60	1 (0.90%)	1 (0.90%)	0 (0%)	0 (0%)	1 (0.90%)	0 (0%)
61 and above	0 (0%)	2 (1.81%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

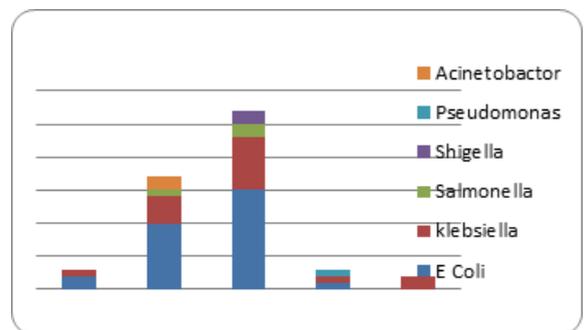


Chart 3:- Age wise distribution of bacterial isolates.

According to gender wise distribution of isolated bacteria in symptomatic cholelithiasis on culture sensitivity, E Coli was isolated in 8 (7.26%) males and 20 (18.18%) females, Klebsiella in 5(5.55%) males and 11(10%) females. 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 shows high resistance to Ampicillin in 17(60.71%) patients followed by resistance to Gentamycine in 12(42.86%) patients. Klebsiella showed high sensitivity to Gentamycine in 13(81.25%), Cefuroxime 12(75%), patients. The resistance of Klebsiella was noted maximum to Ampicillin which was in 10(62.5%) patients followed by resistance to Moxifloxacin in 8(50%). Salmonella showed high sensitivity to Cefuroxime in 3(100%) while the resistance was high to Ampicillin 3(100%) patients. Shigella showed high sensitivity to Gentamycine in 2(100%) and Cefuroxime 2(100%) cases. The resistance of Shigella was noted in maximum to Ampicillin in 2(100%) patients. The only culture grown pseudomonas was sensitive to Cefuroxime & Gentamycine and resistant to Moxifloxacin and Ampicillin. The sensitivity of acinetobacter was high in Cefuroxime 2(100%), Gentamycine 2 (100%) & Ceftriaxone 2(100%), both the Acinetobacter growth were resistant to Ampicillin & Moxifloxacin. Sensitivity and resistance of these 6 bacteria to various antibiotics is shown in detail in table 3.

Discussion:

In our study on culture and sensitivity test, 52(47.28%) have positive growth while 58(52.73%) has no growth. In different studies, the bacterial growth in bile culture was found at the rates of 16-60%. The most common bacteria isolated in our study was E Coli 28(25.45%) followed by Klebsiella 16(14.54%) Salmonella 3(2.73%), Shigella 2(1.81%), Pseudomonas 1(0.90) and Acinetobacter 02(1.81%).

In a study by Capoor et al 104 patients were studied. The patients were divided into three groups: Group A consisted of patients with acute cholecystitis with cholelithiasis; Group B consisted of patients with acute cholecystitis with gastrointestinal ailments requiring biliary drainage and group C consisted of patients with gallbladder carcinoma. Gallbladder, bile and gallstones were subjected to complete microbiological and histopathological examination. Bacteria were recovered from 17 samples (32%) in Group A, 17 (51.4%) in Group B and 1 (1.6%) in Group C. The most common organisms isolated were Escherichia Coli (11, 29.7%), Klebsiella (10, 27%), Clostridium (3, 8.1%), Salmonella Enterica serovar Typhi (3, 8.1%), etc. The majority of Enterobacteriaceae isolates were susceptible to piperacillin-tazobactam and meropenem. As regards Salmonella spp., S Typhi was isolated from 2 (3.8%) patients in Group A and 1 (16%) in Group C 44.

In a study by Ozturk et al, 114 patients who underwent cholecystectomy for various reasons were included in the study. Bacterial growth was isolated in the bile culture of 15 patients (13.1%). The most commonly isolated bacteria were Enterococcus species (4 pts, 26.6%), E Coli (3 patients, 20%) and Enterobacter spp (3 patients, 20%). The bile culture positivity rate was highest in patients with acute cholecystitis combined with choledocholithiasis (3 patients, 100%). The bile culture bacterial growth was highest in patients over 60 years of age (10 patients, 27%) and those with concomitant illness (9 patients, 23.6%).

In our study, on culture and sensitivity test, E Coli shows high sensitivity to Cefuroxime in 22(78.57%) cases followed by Ceftriaxone in 21 (75.00%) patients. E coli shows high resistance to Ampicillin in 17(60.71%) patients followed by resistant to Gentamycine in 12 (42.86%) patients. Klebsiella shows high sensitivity to both Gentamycine in 13(81.25%) and Cefuroxime in 12(75%) patients. The resistant of Klebsiella was noted maximum to Ampicillin which was in 10(62.5%) patients followed by resistant to Moxifloxacin in 8(50%).

Salmonella shows high sensitivity to Cefuroxime in 3(100%) & Gentamycine in 3(100%) while the resistance was high to Ampicillin 3(100%) patients. Shigella showed high sensitivity to

Gentamycine in 2(100%) & Cefuroxime in 2(100%) cases. The resistance of Shigella was noted in maximum to Ampicillin in 2(100%) patients. Pseudomonas showed high sensitivity to Cefuroxime and Gentamycine & resistance to Moxifloxacin and Ampicillin. Acinetobacter showed 100% sensitivity to gentamycine, Cefuroxime and Ceftriaxone & resistance to Ampicillin and Moxifloxacin.

In our series of patients, majorities of isolates were susceptible to Cefuroxime, Gentamycine and Ceftriaxone and were resistant to Ampicillin. As regards to S Typhi these were susceptible to Cefuroxime. This is despite the fact that there are increasing reports of resistance to these drugs from Indian subcontinent. It seems that history of previous and recurrent hospitalization, prolong hospital stay and wide spread use of broad spectrum antibiotics has led to the selective survival and emergence of resistant organism. Therefore antimicrobial activity against potential causative organisms, the severity of cholecystitis, and the local susceptibility pattern must be taken into consideration when prescribing drugs. Prior studies have observed excellent responses with Piperacillin Tazobactam and Meropenem with quinolones for gram negative isolates and Vancomycin for gram positive isolates being preferred.

Therefore antimicrobial activity against potential causative organisms, the severity of cholecystitis, and the local susceptibility pattern must be taken into consideration when prescribing drugs.

Conclusions:

The most common bacteria of symptomatic Cholelithiasis isolated were E. Coli followed by Klebsiella, Salmonella and Shigella. These bacteria show maximum sensitivity to Cefuroxime, Ceftriaxone and Gentamycine. The empirical antibiotics used for the treatment of symptomatic cholelithiasis must cover these common bacteria. Cefuroxime or/and Ceftriaxone and Gentamycine must be a part of empirical regime as it will help in reducing the morbidity associated with symptomatic cholelithiasis.

References:

- Ma'ire Begley a, Cormac G.M. Gahan, Colin Hill. The interaction between bacteria and bile. *FEMS Microbiology Reviews* 29 (2005) 625-651
- Swidsinski A(1), Lee SP. The role of bacteria in gallstone pathogenesis. *Front Biosci*. 2001 Oct 1;6:E93-103.
- Dooley, Hamilton-Miller, Brumfitt, and Sherlock. Antibiotics in the treatment of biliary infection. *Gut*, 1984, 25, 988-998
- Raphael Reiss, Avinoam Elashiv, Alexander A. Septic complications & bile cultures in 800 consecutive cholecystectomies. *World journal of Surgery* vol 6. No 2, March 1982, pp 195-198.
- Sahu MK.A Chako. Dutta AK. Microbial profile and Antibiotic sensitivity pattern in acute bacterial cholangitis. *Indian Gastroenterol* (Sep-Oct 2011) 30(5):204-208.
- Shivprakash S, Harish R, Dinesh KR, Karim PM. Aerobic bacterial isolates from choledochal bile at a tertiary hospital. *Indian journal of Pathology & Microbiology* [2006.49(3)464-467]
- Mahsim kaya, Remzi bestas, Fatma becalan, Farhat bacasiz, EG Arslan. Microbial profile and Antibiotic sensitivity pattern in bile cultures from endoscopic retrograde Cholangiography patients. *World journal of Gastroenterology*. July 21 2012 .18.(17)3585-3589.
- Nasir Mahmud Wattoo. Frequency of infected bile in patients of uncomplicated gallstone disease. *Pakistan Armed forces med journal*. June 2010. Issue no 2.
- Rakesh Neve, S Biswas, V Dhir, KM Mohandas, Rohi Kelkar, Parul Sukla. Bile culture and sensitivity patterns in malignant obstructive jaundice. *Indian journal of Gastroenterology* 2003 vol 22 Jan-Feb 22 (1) 16-8. 12617446.
- Vasitha Abeysuriya, Kemal Ismil Deen, Tamara Wijesuriya, Sujatha Senadera Salgado. Microbiology of gall bladder bile in uncomplicated symptomatic Cholelithiasis. *Hepatobiliary pancreatic disease int* vol 7 No 6 2008.
- Salvador VB, Lozada MC, Consunji RJ. Microbiology and antibiotic susceptibility of organisms in bile cultures from patients with or without cholangitis at an Asian academic medical centre. *Surgical Infection*. Larchet. 2011 Apr; 12(2): 105-11. Epub 2011 Feb 24.
- Kanafani JA, Khalif N, Kani SS, Araj GF, Khalifeh M, Sharara AI. Antibiotic use in acute cholecystitis: Practice patterns in absence of evidence based guidelines. *J Infect* 2005 Aug; 51:128-34. Epub 2005 Jan
- Sheen-Chan S, Chen W, Enq H, Sheen C, Chou F, Cheng Y, Lee T. Bacteriology and antimicrobial choice in hepatolithiasis. *Am J Infect Control*. 2000 Aug; 28(4):298-301.
- M Ballal, KN Jyothi, B Anthony, C Arun, T Prabhu, PG Shivananda. Bacteriological spectrum of cholecystitis and its antibiogram (2001). *Indian journal of Medical Microbiology*. vol 19. (4). 212-214.
- M Al Harbi, A O Osaba, A Mowallad. Tract microflora in Saudi patients with cholelithiasis. *July 2001. Tropical Medicine of International health* volume 6 no 7 pp 570-574.
- Chang WT, Lee KT, Wang SR, Chuang SC, Kuo KK, Chen JS, Sheen PC. Bacteriology and antimicrobial susceptibility in biliary tract disease: An audit of 10 years experience. *Kaohsiung j of med science*. 2002 May; 18(5):221-8.
- C Sendes A, Burdiles P, Maluenda F, Diaz AC, Sendes P, Mitru N. Simultaneous bacteriological assessment of bile from gallbladder and common bile duct in control subjects and patients with gallstones and common duct stones. *arch*

- Surgery. 1996 Apr;131(4):389-94.
18. Neqm AA, Scott A. Routine bile collection for microbiological analysis during cholangiography and its impact on management of cholangitis. *Gastrointestinal endoscopy* Aug 2011. vol(5) 204-215.
 19. Jeong Seok Kwon, Jimin Han, Tae Won Kim, Ji-Hye Oh, Hyun Hee Kwon, Jin Tae Jung, Joong Goo Kwon, Eun Young Kim and Ho Gak Changes in Causative Pathogens of Acute Cholangitis and Their Antimicrobial Susceptibility over a Period of 6 Years *Korean J Gastroenterol* Vol. 63 No. 5, 299-307
<http://dx.doi.org/10.4166/kjg.2014.63.5.299>
 20. Michael J, Englesbe, Lillian G Dawes. Resistant pathogen in Biliary obstruction: Importance of cultures to guide antibiotic therapy. *HPB*(2005); 7; 144-148.
 21. Phillipo L Chalya , Emmanuel S Kanumba and Mabula Mchembe2† Etiological spectrum and treatment outcome of Obstructive jaundice at a University teaching Hospital in northwestern Tanzania: A diagnostic and therapeutic challenges
<http://www.biomedcentral.com/1756-0500/4/147>
 22. Emin Turk 1, Erdal Karagulle 1, Kivanc Serefhanoglu 2, Hale Turan 2, Gokhan Moray Effect of Cefazolin Prophylaxis on Postoperative Infectious Complications in Elective Laparoscopic Cholecystectomy: A Prospective Randomized Study. *Iranian Red Crescent Medical Journal*. 2013 July; 15(7): 581-6.
 23. Irfan Sattar, Adnan Aziz, Shahid Rasul, Zahid Mehmood and Asadullah Khan . Frequency of infection in Cholelithiasis. *JCPSP* 2007, Vol. 17 (1): 48-50.
 24. Thawee Ratanachu-ek, Pitchaya Prajanphanit, Kawin Leelawat, Suchart Chantawibul, Sukij Panpimanmas, Role of ciprofloxacin in patients with cholestasis after Endoscopic retrograde cholangiopancreatography. *World J Gastroenterol* 2007 January 14; 13(2): 276-279 . www.wjgnet.com. *World Journal of Gastroenterology* ISSN 1007-9327
 25. S Baliga, S Gopal, A Jain, SM Shenoy, S Shenoy, BV Tantry. Clinicomicrobiological analysis of patients with cholangitis. *Indian journal of medical microbiology*. vol 32, No 2, April-June, 2014. pp 157-160.
 26. Sashidhar k nagraj, Prasad Paul, Mohan k Kumar, S Munenarayanappa. Risk factor and biochemical evaluation of biliary calculi in rural Kolar, Karnataka, India : A rural perspective of an urban disease. *Journal of Clinical and diagnostic research*. 2012 May; 6: 364-368.
 27. H Ohdan, H Oshiro, Y Yamamoto, I Tanaka, K Inagaki, K Sumimoto. Bacteriological investigation of bile in patients with cholelithiasis. *Surgery today*. Japan *J. Surgery*(1993).23:390-395.
 28. G.J. Morris-Stiff, P. O'Donohue, S. Ogunbiyi & W.G. Sheridan Microbiological assessment of bile during cholecystectomy: is all bile infected? *HPB*, 2007; 9: 225-228.
 29. Abd-Alnabi Jwaied Abid Sama Jwad Kadhim. Bacteriological and Immunological study of Cholecystectomy patients. *Journal of Babylon University/Pure and Applied Sciences/No.(2)/Vol.(22)*: 2014.
 30. Bahman Darkahi. Biliary microflora in patients undergoing cholecystectomy *J Bacteriol Parasitol* 2013; 4:4 <http://dx.doi.org/10.4172/2155-9597.S1.004>
 31. Mohammad Moazeni Bistgani , Reza Imani. Bacteria Isolated From Patients With Cholelithiasis and Their Antibacterial Susceptibility Pattern. *Iranian Red Crescent Medical Journal*. 2013 August; 15(8): 759-61.
 32. Mir MA(1), Malik UY, Wani H, Bali BS. Prevalence, pattern, sensitivity and resistance to antibiotics of different bacteria isolated from port site infection in low risk patients after elective laparoscopic cholecystectomy for symptomatic cholelithiasis at tertiary care hospital of Kashmir. *Int Wound J*. 2013 Feb; 10(1):110-3.
 33. Roa I, Ibacache G, Carvallo J, Melo A, Araya J, De Aretxabala X, Figueroa M, Barrientos F, Figueroa C. Microbiological study of gallbladder bile in a high risk zone for gallbladder cancer. *Rev Med Chil*. 1999 Sep; 127(9):1049-55.
 34. Truedson H, Elmros T, Holm S. The incidence of bacteria in gallbladder bile at acute and elective Cholecystectomy. *Acta Chir Scand*. 1983; 149(3):307-13.
 35. Hancke E, Nutsche A, Marklein G. Bacteria in the gallbladder wall and gallstones--indications for cholecystectomy *Langenbecks Arch Chir*. 1986; 368(4):249-54.
 36. Maluenda F(1), Csendes A, Burdiles P, Diaz J. Bacteriological study of choledochal bile in patients with common bile duct stones, with or without acute suppurative cholangitis. *Hepatogastroenterology*. 1989 Jun; 36(3):132-5.
 37. Yeom DH(1), Oh HJ, Son YW, Kim TH. What are the risk factors for acute suppurative cholangitis caused by common bile duct stones? *Gut Liver*. 2010 Sep; 4(3):363-7.
 38. Stewart L(1), Oesterle AL, Erdan I, Griffiss JM, Way LW. Pathogenesis of pigment gallstones in Western societies: the central role of bacteria. *J Gastrointest Surg*. 2002 Nov-Dec; 6(6):891-903; discussion 903-4.
 39. P Hazra, KTH Oahn, M Tewari, AK Pandey, K Kumar, TM Mohapatra. The frequency of live bacteria in gall stones. *HPB* 2004. Vol 6, No 1, 28-32.
 40. Stewart L(1), Griffiss JM, Jarvis GA, Way LW. Biliary bacterial factors determine the path of gallstone formation. *Am J Surg*. 2006 Nov; 192(5):598-603.
 41. E. Maseda, a G. Maggi, a R. Gomez-Gil, b G. Ruiz, b R. Madero, c A. Garcia-Perea, b L. Aguilar, d F. Gilsanz, a J. Rodriguez-Baño. Prevalence of and Risk Factors for Biliary Carriage of Bacteria Showing Worrisome and Unexpected Resistance Traits. *Journal of Clinical Microbiology* p. 518–521 February 2013 Volume 51 Number 2.
 42. Matin MA, Kunitomo K, Yada S, Miyoshi Y, Matsumura T, Komi N. Biliary stones and bacteriae in bile study in 211 consecutive cases. *Tokushima J Exp Med*. 1989 Jun; 36(1-2):11-6.
 43. Moshahiro y. Tadahiyo T. Yoshifumi K. Atshusi T. Yuji N. Yuji N et al. Antimicrobial therapy for acute cholecystitis: Tokyo guidelines. *J Hepatobiliary pancreat surg*. 2007 January; 14(1): 83-90.
 44. Bae Wk, Moon YS, Kim JH. Microbiologic study of the bile culture and antimicrobial susceptibility in patients with biliary tract infection. *Korean journal of Gastroenterol*. Apr, 51(4):248-54.
 45. Malini R. Capoor1, Deepthi Nair1, Rajni2, Geetika Khanna2, S.V. Krishna3, M.S. Chintamani3 and Pushpa Aggarwal2 Microflora of Bile Aspirates in Patients With Acute Cholecystitis With or Without Cholelithiasis: A Tropical Experience. *The Brazilian Journal of Infectious Diseases* 2008; 12(3):222-225.
 46. Alaattin Ozturk, Hakan Bozkurtoglu, Cengiz Kaya, Necati Tan, Hülya Cascurlu, Ömer Faruk Akinci. Bacteriologic Analysis of Bile in Cholecystectomy Patients. *The New Journal of Medicine* 2012; 29(1):43-46.
 47. Velaquez-Mendoza JD, Alvarez-Mora M, Velaquez-Morales CA, Anaya-Prado R. Bactibilia and surgical site infection after open cholecystectomy. *Cir Cir* 2010; 78:239-43.
 48. N Suna, H Yildiz, M Yuksel, E Parlak, S Disibeyaz, B Odems. The change in microorganisms reproducing in bile and blood culture and antibiotic susceptibility over the years. *Turk J Gastroenterol* 2014; 25: 284-90.