

FREQUENCY OF COMMON BACTERIA AND THEIR ANTIBIOTIC SENSITIVITY IN PATIENTS WITH SYMPTOMATIC CHOLELITHIASIS

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ABSTRACT

Objective: To determine the frequency of common bacteria and their antibiotic sensitivity in patients with symptomatic cholelithiasis

Methodology: This cross sectional descriptive study was conducted at Department of Surgery, Lady reading hospital, Peshawar for 6 months i.e., from December 2012 to May 2013. The total number of patients were 126. They presented with symptomatic cholelithiasis. Bile was aspirated peroperatively before cholecystectomy from gall bladder and was sent for culture and sensitivity test. The data was analyzed using Statistical Package for Social Sciences (SPSS) version 10.0.

Results: On culture and sensitivity test, 74 (58.73%) have positive growth while 52 (41.27%) have no growth. The most common bacteria was E. Coli isolated in 28 (22.22%) patients followed by Klebsella in 22 (17.46%), Salmonella in 16 (12.70%) and Shigella in 8 (6.35%) patients. On culture and sensitivity test, all the 4 isolated bacteria showed sensitivity to Cefuroxime, Ceftriaxone and Ciprofloxacin in more than 50 % cases, while all the four bacteria showed resistance to amoxicillin in more than 50 % cases.

Conclusion: The most common bacteria of symptomatic cholelithiasis are E. coli and Klebsella followed by Salmonella and Shigella. These bacteria showed maximum sensitive to cefuroxime and ceftriaxone.

Key Words: Symptomatic cholelithiasis, Culture and sensitivity, Antibiotics.

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INTRODUCTION

Gallstone disease (GD) is a common problem in elderly women and there has been a very well known association of this disease with obesity and nulliparity. The disease has been found very infrequently in children¹.

Although this disease has a low mortality rate, its economic and health impact is significant due to its high morbidity. In fact, GD is one of the most common abdominal conditions for which patients in developed countries are admitted to hospitals and this frequency has increased in Western countries since the 1950s. However, since the introduction of laparoscopic cholecystectomy in the early 90s, which is considered a safe treatment for GD, a possible unjustified increase in surgical procedures has been observed. Therefore, there is the need for more knowledge of the epidemiological characteristics of GD in order to better identify therapeutic strategies.

The availability of ultrasonography (US) as an accurate tool for gallstone diagnosis has allowed the evaluation of gallstone prevalence by means of epidemiological surveys of the general population, both in Eastern and Western countries. Furthermore, these studies, as well as case-control studies, have allowed the identification of the factors most frequently associated with GD, i.e. increasing age, female sex, familial history of GD, number of pregnancies, obesity, or type 2 diabetes². Cholelithiasis is an important cause of morbidity throughout the world³.

The incidence of symptomatic cholelithiasis is reported to be 2.2/1000 USA population with more than 500,000 cholecystectomies performed yearly⁴.

Among different factors causing gallstones formation, biliary infection can be found in a sizeable proportion of patients. Biliary infection can be due to gram negative, gram positive or anaerobic organisms⁵.

Gallstones cause various problems besides simple biliary colic and cholecystitis. With chronicity of inflammation caused by gallstone obstruction of the cystic duct or the gallbladder may fuse to the extrahepatic biliary tree, causing Mirizzi syndrome, or fistulize into the intestinal tract, causing so-called gallstone ileus. Stones may pass out of the gallbladder and travel downstream through the common bile duct to obstruct the ampulla of Vater resulting in gallstone pancreatitis, or pass out of the gallbladder inadvertently during surgery, resulting in the syndromes associated with lost gallstones⁶.

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 bactibilia⁷.

In a study from Karachi, out of 100 patients undergoing cholecystectomy 36(36%) patients were having bactibilia⁵.

Gomes et al reported a prevalence of bactibilia in 20 (20%) patients with organisms such as E.coli (40%), Klebsiella (35%), Salmonella (20%) and Shigella (20%) who underwent cholecystectomy⁷.

In another study from United Kingdom, 20 (15.6%) out of 128 patients were found to have culture detected microorganisms⁸.

The pathogenesis of bile infection is incompletely understood, with the prevailing theories not fully explaining all the observations⁹⁻¹¹.

There is relatively sparse data, both local & international on the prevalence of the infection in patients undergoing cholecystectomy⁸.

The conservative & prophylactic treatment therefore is based on best guess basis⁷.

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 results of this study will be shared with all surgeons and general practitioners in the periphery to help them identify the type of antibiotic to be administered to patients with symptomatic cholelithiasis before referring them to tertiary care. This will help us in reducing the morbidity associated with cholelithiasis.

METHODOLOGY

The descriptive cross sectional study was carried out at Surgical "C" unit, Govt. Lady Reading Hospital, Peshawar. The duration of study was six months from

1st December, 2012 to 30th May, 2013. Non probability (consecutive) sampling technique was used and a total of 126 patients were included in study. This sample size was calculated by using 20%⁷ prevalence of Shigella, 95% confidence interval and 7% margin of error using WHO software for sample size calculation.

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.

The approval for the study was obtained from the Ethical Committee of the Hospital. All the study patients presenting with symptoms (Pain right Hypochondrium, and Vomiting), and sign (Tender right Hypochondrium) were admitted in surgical B unit 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 anaesthesia fitness like chest X-ray, ECG and Liver function tests were performed. The purpose and procedure of the study were explained to the patients and a written informed consent was obtained.

All the patients were operated through open cholecystectomy on the next elective list by a single consultant surgeon. All patients were given an IV injection of cefuroxime 1.5 gram at induction of anaesthesia and 2 doses of the same were repeated postoperatively. After opening on 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.

The collected specimen of the bile was labeled and sent to a single laboratory in 5cc disposable syringe. Both aerobic & anaerobic cultures of specimen were performed for microorganisms such as E.coli, Klebsiella, Salmonella and Shigella under the supervision of expert microbiologist. For aerobic culture, the sample was inoculated on blood agar and MacConkey agar medium and incubated at 37C for 24 hours. For anaerobic culture, the sample was inoculated on blood agar medium with a Metronidazole disc between primary and secondary streak lines. Once detected the sensitivity of these bacteria was checked for antibiotics like cefradine, cefuroxime, ceftriaxone, ciprofloxacin and amoxicillin.

Patient demographics like age, gender and culture reports of bile were recorded in a structured proforma.

The data was analyzed with SPSS version 10 for windows. Frequency and percentages were calculated from categorical variables like gender, common bacteria such as E.coli, Klebseilla, Salmonella and Shigella and their antibiotic sensitivity while means \pm standard deviation was calculated for continuous variables like age. Common bacteria were stratified among the age and sex to see the effect modifiers and also cross tabulation was used to see the sensitivity pattern of common bacteria to different antibiotics. The data was presented in the form of tables.

RESULTS

The total number of patients presenting with symptomatic cholelithiasis were 126. Out of these, male and female patients were 26 (20.63%) and 100 (79.37%) respectively with male to female ratio of 1:3.85.

The mean age of male and female patients with symptomatic cholelithiasis 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).

On culture and sensitivity test, 74 (58.73%) have positive growth while 52 (41.27%) has no growth.

The most common bacteria isolated was E. Coli 28 (22.22%) followed by Klebseilla 22 (17.46%), Salmonella 16 (12.70%) and Shigella 8 (6.35%).

Maximum number of patients presenting with symptomatic cholelithiasis were 47 (37.30%) that belonged

to the age group of 41 to 50 years followed by 35 (27.78%) from the age groups of 31 to 40 years. As per age wise distribution of isolated bacteria in symptomatic cholelithiasis on culture test of bile, E. Coli was most common in age group of 31 to 40 years; 15 (11.90%), Klebseilla in was common in age group of 41 to 50 years; 10 (7.94%). Full detail of age wise distribution is shown in Table 2.

According to gender wise distribution of isolated bacteria in symptomatic cholelithiasis on culture sensitivity, E. Coli was isolated in 8 (6.35%) males and 20 (15.87%) females, Klebseilla in 5 (3.97%) males and 17 (13.49%) 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 showed high resistance to Amoxicillin in 17 (60.71%) patients followed by resistance to Ciprofloxacin in 12 (42.86%) patients. Klebseilla showed high sensitivity to Ciprofloxacin in 13 (72.22%) patients. The resistance of Klebseilla was noted maximum to Amoxicillin which was in 10 (55.56% patients followed by resistance to Cefradine in 8 (44.44%). Salmonella showed high sensitivity to Cefuroxime in 11 (68.75%) while the resistance was high to Amoxicillin 10 (62.50%) patients. Shigella showed high sensitivity to Ciprofloxacin in 7 (87.50%) cases. The resistance of Shigella was noted in maximum to Amoxicillin in 5 (62.50%) patients. Sensitivity and resistance of these 4 bacteria to various antibiotics is shown in detail in Table 3.

Table 1: Mean age \pm standard deviation of patients with symptomatic Cholelithiasis

Gender	Mean Age \pm Standard Deviation (SD)
Male	46.20 \pm 10.88
Female	45.95 \pm 10.14
Total	46.13 \pm 10.65

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 (22.22%)	Klebseilla n=18 (17.46%)	Salmonella n=16 (12.70%)	Shigella n=8 (6.35%)
18-30	2 (1.59%)	1 (0.79%)	1 (0.79%)	0 (0%)
31-40	10 (7.94%)	6 (4.76%)	5 (3.97%)	2 (1.59%)
41-50	15 (11.90%)	10 (7.94%)	8 (6.35%)	4 (3.17%)
51-60	1 (0.79%)	2 (1.59%)	1 (0.79%)	1 (0.79%)
61 and above	0 (0%)	3 (2.38%)	1 (0.79%)	1 (0.79%)

Table 3: Sensitivity and resistance of common isolated bacteria to various antibiotics on culture and sensitivity test of bile in patients with symptomatic Cholelithiasis

Antibiotic	E. coli n=28 (22.22%)		Klebseilla n=18 (17.46%)		Salmonella n=16 (12.70%)		Shigella n=8 (6.35%)	
	S	R	S	R	S	R	S	R
Cefradine	17 (60.71%)	11 (39.29%)	10 (55.56%)	8 (44.44%)	10 (62.50%)	6 (37.50%)	5 (62.50%)	3 (37.50%)
Cefuroxime	22 (78.57%)	6 (21.43%)	12 (66.67%)	6 (33.33%)	11 (68.75%)	5 (31.25%)	6 (75.00%)	2 (25.00%)
Ceftriaxone	21 (75.00%)	7 (25.00%)	11 (61.11%)	7 (38.89%)	10 (62.50%)	6 (37.50%)	5 (62.50%)	3 (37.50%)
Ciprofloxacin	16 (57.14%)	12 (42.86%)	13 (72.22%)	5 (27.78%)	9 (56.25%)	7 (43.75%)	7 (87.50%)	1 (12.50%)
Amoxicillin	11 (39.29%)	17 (60.71%)	8 (44.44%)	10 (55.56%)	6 (37.50%)	10 (62.50%)	3 (37.50%)	5 (62.50%)

S= Sensitive R=Resistance

DISCUSSION

In our study on culture and sensitivity test, 74 (58.73%) have positive growth while 52 (41.27%) has no growth. In different studies, the bacterial growth in the bile culture was found at the rates of 16-54%¹²⁻¹⁸.

The most common bacteria isolated in our study was E. Coli 28 (22.22%) followed by Klebseilla 22 (17.46%), Salmonella 16 (12.70%) and Shigella 8 (6.35%). In a study by Capoor et al¹⁹, total of 104 bile samples were studied and bacteria were isolated in 37 samples (35.6%). They observed monomicrobial infection in 32 (30.8%). Polymicrobial infection was seen in 5 (4.8%). The most common organisms isolated were Escherichia coli (11, 29.7%), Klebsiella pneumoniae (10, 27%), Citrobacter freundii (3, 8.1%), Salmonella enterica serovar Typhi (3, 8.1%), Pseudomonas aeruginosa (2, 5.4%), Acinetobacter spp. (1, 2.7%), Candida krusei (1, 2.7%), Staphylococcus aureus (1, 2.7%). Polymicrobial infection of P. aeruginosa with K. pneumoniae was observed in 4 patients (3.8%).

In a study by Öztürk et al²⁰, 114 patients who underwent cholecystectomy for various reasons were included in the study. Bacterial growth was detected in the bile culture of 15 patients (13.1%). The most commonly isolated bacteria were Enterococcus spp (4 patients, %26.6%), Escherichia coli (3 patients, 20%) and Enterobacter spp (3 patients, 20%). The bile culture positivity rate was highest in patients with acute cholecystitis combined with choledocolithiasis (3 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 (9 patients, 23.6%). Postoperative surgical site infection was detected in only one patient; there were no surgical site infections in patients with a positive bile culture. In another study, Bacteria isolated in gallbladder bile culture were E. coli (30%), Enterobacter sp. (15%), Staphylococcus aureus (10%), Streptococcus faecalis (15%), Klebsiella (5%), Serratia (2.5%), Streptococcus (2.5%), Streptococcus sp (20%)²¹.

In a study, Bile specimens were obtained by syringe aspiration from common bile duct in 150 patients with hepatolithiasis who underwent surgical intervention²². Bacteria were present in the bile of all patients. The bacteria most frequently found were gram-negative bacteria such as Klebsiella sp, Escherichia coli, and Pseudomonas sp, and the gram-positive Enterococcus sp. Bacteroides sp were the most frequently found anaerobes.

Abeyasuriya et al¹⁴, performed a case control study of 70 bile samples (35 cholesterol and 35 pigment stones from 51 females and 19 males) from patients who underwent laparoscopic cholecystectomy for uncomplicated cholelithiasis, and 20 controls (14 females and 6 males, aged 33-70 years with a median age of 38 years) who underwent laparotomy and had no gallbladder stone shown by ultrasound scan. The bile samples were aerobically cultured to assess microflora and their antibiotic susceptibility. 38(54%) of the 70 patients with gallstones had bacterial isolates. 9 isolates (26%) were

from cholesterol stone-containing bile and 29 isolates (82%) from pigment stone-containing bile ($P=0.01$, t test). Twenty-eight of these 38 (74%) bile samples were shown positive only after enrichment in brain heart infusion medium (BHI) ($P=0.02$, t test). The overall bacterial isolates from bile samples revealed *E. coli* predominantly, followed by *P. aeruginosa*, *Enterococcus* spp., *Klebsiella* spp. and *S. epidermidis*. There were no bacterial isolates in the bile of controls after either direct inoculation or enrichment in BHI.

In a study by Ballal et al²³, a total of 125 bile samples along with 25 gall stones were processed for both aerobic and anaerobic microorganisms. Bile cultures grew bacteria in 88(70.4%) of 125 patients out of which 71(56.8%) were aerobes and the remaining 17(13.6%) were anaerobes. Mixed bacterial flora was seen in 7 cases. Among the mixed flora, 2 had only aerobes and the remaining 5 had both aerobes and anaerobes in them. Of the 25 gall stones processed, 6 yielded growth of aerobic bacteria which were similar to the isolates in bile cultures from the same patients. All cultures were negative in the control group. Analysis of the bacterial flora showed that *Escherichia coli* was the most common isolate both in bile as well as in gall stones which was isolated either singly or in association with other organisms in clinical specimens. *Salmonella typhi* was isolated from 2 bile samples followed by *Klebsiella*. Maximum isolates 34(45.4%) were seen in age groups between 51-60 years.

Although surgical intervention remains the mainstay of therapy for acute cholecystitis and its complications, however before elective or emergency cholecystectomy a period of hospitalization is required. In the current study, the empirical antibiotics were given according to recommended guidelines^{24, 25} and these were changed as culture and sensitivity results were available. As post-operative complication of wound infection, abscess formation or sepsis are reduced in antibiotic treated patients. In brief, for mild cases of biliary colic, the administration of non-steroidal anti-inflammatory drugs (NSAIDs) is recommended to prevent progression of inflammation (recommendation grade A). For moderate infection, agents with a narrow spectrum of activity such as cefuroxime or ciprofloxacin plus metronidazole are preferred. For severe infections, combination drugs or carbapenem are recommended. The latter also required hydration and electrolyte correction and elimination of oral intake. In our study, on culture and sensitivity test, *E. coli* showed high sensitivity to Cefuroxime in 22 (78.57%) cases followed by Ceftriaxone in 21 (75.00%) patients. *E. coli* showed high resistance to Amoxicillin in 17 (60.71%) patients followed by resistance to Ciprofloxacin in 12 (42.86%) patients. *Klebsiella* showed high

sensitivity to both Ciprofloxacin in 13 (72.22%) patients. The resistance of *Klebsiella* was noted maximum to Amoxicillin which was in 10 (55.56% patients followed by resistance to Cefradine in 8 (44.44%). *Salmonella* showed high sensitivity to Cefuroxime in 11 (68.75%) while the resistance was high to Amoxicillin 10 (62.50%) patients. *Shigella* showed high sensitivity to Ciprofloxacin in 7 (87.50%) cases. The resistance of *Shigella* was noted in maximum to Amoxicillin in 5 (62.50%) patients.

In our series of patients, majorities of isolates were susceptible to Cefuroxime and ceftriaxone and were resistant to amoxicillin. As regards, *S. Typhi*, these were all susceptible to ciprofloxacin and ceftriaxone. This is despite the fact that there are increasing reports of resistance to these drugs from the 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^{26, 27}. Therefore, antimicrobial activity against potential causative organisms, the severity of the 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^{5, 28, 29}.

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

CONCLUSION

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

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CONTRIBUTORS

FM planned the study and wrote manuscript. MAK and AF helped in data analysis and manuscript writing. MK supervised the study. All authors contributed significantly to the final manuscript.