

# COMMON BACTERIOLOGICAL PATHOGENS AND THEIR ANTIBIOTIC SENSITIVITY PATTERN IN BILE OF PATIENTS WITH CHOLELITHIASIS

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## ABSTRACT

**Background:** To determine the pattern of antibiotic susceptibility of common bacteriological pathogens of bile in patients with Cholelithiasis.

**Material and Methods:** A prospective cross-sectional study was conducted in the department of surgery, Jinnah Postgraduate Medical Center, from January 2020 to July 2020. All patients between the ages of 16 and 65, with symptomatic cholelithiasis operated by open or laparoscopic cholecystectomy were included in this study. Patients with acute cholecystitis, obstructive jaundice, and common bile duct stones were excluded from the study. Cultures and Sensitivity tests were performed for aerobes and anaerobes pathogens in JPMC laboratory. Socio-demographic variables and clinical parameters were recorded in a predefined proforma. Data was entered and analyzed using Statistical Package for Social Sciences version 26.

**Results:** Out of a total of 610 samples, 314 cultures were positive for bacteria. Bacteriological investigation revealed *Escherichia coli* was isolated in 97 (30.89%) patients, *Escherichia coli* and *Klebsiella pneumoniae* in 66 (21.02%), *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella sonnei* in 18 (5.73%), *Escherichia coli*, *Salmonella enterica* in 18 (5.73%), only *Klebsiella pneumoniae* in 64 (20.38%), *Salmonella enterica* in 15 (4.78%), *Salmonella enterica* and *Klebsiella pneumoniae* in 32 (10.19%), and *Shigella sonnei* in 4 (1.27%) patients. The most susceptible antibiotic for microorganisms on the whole was Imipenem (66.7%). The highest resistance was shown against Cefradine (76.4%).

**Conclusion:** The current study concludes that the most common bacteria of symptomatic cholelithiasis are *Escherichia coli* and *Klebsiella pneumoniae* followed by *Salmonella enterica* and *Shigella sonnei*. These bacteria showed an overall susceptibility to Imipenem.

**Keywords:** Bacteriological investigation, Symptomatic cholelithiasis, Culture, Sensitivity, Antibiotics

## BACKGROUND

Bile is a necessary fluid required by the body since it consists of nutrients such as proteins, phospholipids, cholesterol and bile acids. It is produced by the liver but the gallbladder stores it. Bile helps in the absorption of fat in the intestines.<sup>1</sup> The gallbladder is supplied by the cystic artery which comes from the right hepatic artery.<sup>2</sup>

However, sometimes developmental problems are seen in the gallbladder such as multiple gallbladders, agenesis, bi-lobed and double cystic.<sup>3,4</sup> Double cystic gallbladder may be seen with its own cystic duct or the cystic duct may be in the form of a common cystic duct.<sup>5</sup> 14 to 30% of cholecystectomies are done as a

result of acute cholecystectomies.<sup>6,7</sup> Often, certain bacteria infect the gallbladder which is minimized by a healthy function of bile which excretes out harmful organisms. However, this function is limited when stones are developed in the gallbladder or in the common bile duct.<sup>8,9</sup> Gallstones are a common finding in individuals, 7.9% of men in the United States were found to be diagnosed with gallstones and 16.6% of women during a recent survey.<sup>9,10</sup>

More research is required to identify the reasons for sensitivity of certain bacteria towards antibiotics in patients who present symptomatically with cholelithiasis in order to reduce mortality and morbidity. Due to limited data from the local population, the current study was undertaken.

## MATERIAL AND METHODS


A prospective cross-sectional study was conducted at the department of surgery, Jinnah Postgraduate Medical Center, from January 2020 to July 2020. Ethical approval was obtained from the institutional review board of JPMC. A non-randomized consecutive sampling technique was applied to enroll the

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participants in the study. The frequency of common bacteria i.e. *Shigella sonnei* was 6.35% [11] in patients with cholelithiasis at margin of error 4% with confidence interval 95%. After putting the values in the formula  $n = (Z_{1-\alpha/2})^2 P q / d^2$  sample size was =143 no. of specimens using WHO software for sample size calculation.

All patients between the ages of 16 and 65 year with symptomatic c+66holelithiasis operated by open or laparoscopic cholecystectomy were included in this study. All patients gave informed verbal and written consent to take part in the study. Patients with acute cholecystitis, obstructive jaundice, and common bile duct stones were excluded from the study. Cholecystitis was suspected in patients with TLC count greater than 12000 WBCs per microliter, high-grade fever, tenderness in the right upper quadrant.

Once patients were diagnosed with cholelithiasis on ultrasound imaging, they underwent surgery or laparoscopic cholecystectomy and then specimens from bile were sent for microbiological evaluation in sterile syringe. Patients were prescribed first prescribed empirical antibiotics postoperatively then changed the antibiotics according to the bacteria isolated. Duration of antibiotics varied from five to seven days. During the surgery, operative findings were documented and bile was aspirated in a 5-milliliter disposable and sterile syringe. Gallbladder was excised after cystic artery and duct were ligated while in laparoscopic cholecystectomy sample collected in 5cc syringe, after removing gallbladder from abdominal cavity. The collected specimen of the bile was labeled and sent to a JPMC laboratory. Sociodemographic (Age and gender) variables and clinical parameters (duration of hospital stay, duration of fever and total number of doses used) were recorded in a predefined proforma.

Data was entered and analyzed using Statistical Package for Social Sciences version 26. Common bacteriological investigation and their antibiotic sensitivity pattern in bile, in terms of drug sensitivity or susceptibility, was the unit of analysis. Descriptive statistics of age, duration of hospital stay and duration of fever, were computed in terms of Mean  $\pm$  SD and calculated. Frequency and percentages were also computed for gender. Family history of cholelithiasis, surgical approach (Laparoscopic/ Open cholecystectomy), were also recorded. Effect modifier was controlled through stratification like age, gender,

duration of hospital stays, and surgical approach to see the impact on of common bacteriological investigation and their antibiotic sensitivity pattern. Appropriate Chi-square test or Fisher exact test was applied. Two-sided tailed test at 95% confidence interval was applied at 5% level of significance was taken as significance.

## RESULTS

A total of 610 samples were studied out of these, 314 (51.47%) cultures were positive for bacteria. The mean age of patients was  $45.2 \pm 8.6$  years. The mean duration of hospital stay was  $2.5 \pm 1.3$  days and the mean duration of antibiotic treatment was  $2.5 \pm 1.1$  days (2.33 - 2.70). There were 81 (25.80%) male and 233 (74.20%) female patients. See Table-II for socio-demographic and clinical characteristics. Laparoscopic surgery was done in 108 patients (75.5%) while 35 (24.5%) were managed via open cholecystectomy.

Bacteriological investigation revealed *Escherichia coli* was isolated in 97 (30.89%) patients, *Escherichia coli* and *Klebsiella pneumoniae* in 66 (21.02%), *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella sonnei* in 18 (5.73%), *Escherichia coli*, *Salmonella enterica* in 18 (5.73%), only *Klebsiella pneumoniae* in 64 (20.38%), *Salmonella enterica* in 15 (4.78%), *Salmonella enterica* and *Klebsiella pneumoniae* in 32 (10.19%), and *Shigella sonnei* in 4 (1.27%) patients (Table-III).

The most susceptible antibiotic for microorganisms on the whole was Imipenem (66.7%). The highest resistance was shown against Cefradine (76.4%). The overall susceptibility pattern of the isolates is summarized in Table-IV.

**Table-I: Mean age, hospital stay, and duration of fever among study participants.**

Variables	Mean (standard deviation)
Age (years)	45.23 $\pm$ 8.67
Hospital stay (days)	2.59 $\pm$ 1.33
Duration of Fever (days)	2.68 $\pm$ 1.29

**Table-II: Characteristics of patients in the study**

Variables	N (percentage)
<b>Gender</b>	
Male	81 (25.80%)
Female	233 (74.20%)
<b>Family History of Cholelithiasis</b>	
Positive	248 (78.98%)

Negative	66 (21.02%)
<b>Surgical approach</b>	
Laparoscopic	237 (75.48%)
Cholecystectomy	77 (24.52%)

**Table-III: Bacteriological pathogens (n=314).**

Bacteriological Pathogen	N (%)
<i>Escherichia coli</i>	97 (30.89%)
Both <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> isolated	66 (21.02%)
Mixed infection ( <i>Escherichia coli</i> + <i>Klebsiella pneumoniae</i> + <i>Shigella sonnei</i> )	18 (5.73%)
<i>Escherichia coli</i> + <i>Salmonella enterica</i>	18 (5.73%)
<i>Klebsiella pneumoniae</i> only	64 (20.38%)
<i>Salmonella enterica</i> only	15 (4.78%)
<i>Salmonella enterica</i> + <i>Klebsiella pneumoniae</i>	32 (10.19%)
<i>Shigella sonnei</i> only	4 (1.27%)

**Table-IV: Antimicrobial susceptibility patterns in the study population**

Antimicrobial Susceptibility Patterns	Resistant	Sensitive
Cefradine zone diameter > 16	13 (76.4%)	4 (23.5%)
Cefuroxime zone diameter > 30	44 (58.6%)	31 (41.3%)
Ceftriaxone zone diameter > 30	33 (62.2%)	20 (37.7%)
Ciprofloxacin zone diameter > 25	64 (58.1%)	46 (41.8%)
Amoxicillin zone diameter > 30	24 (64.8%)	13 (35.1%)
Cefoxitin (FOX) zone diameter > 22	9 (56.25%)	7 (43.75%)
Imipenem (IMP) zone diameter > 10	2 (33.3%)	5 (66.7%)

## DISCUSSION

Literature has shown that gram negative bacteria are the most frequently isolated pathogens from bile, specifically *Escherichia coli*.<sup>12-13</sup> Due to the increasing frequency of multidrug-resistant Gram-negative pathogens, the preference of appropriate antibiotics is limited. Unnecessary and inadequate use of antibiotics, are two most important factors contributing to the current resistance patterns. Thus, it is important to be aware about the common pathogens and their sensitivity and susceptibility patterns in the local population. This is to ensure that patients are receiving the most optimum empirical antibiotic therapy, postoperatively.

In the present study, we found that the majority of the patients were in their forties. This is according to the

study conducted by Manan *et al.*,<sup>11</sup> who reported a mean age of 45.95±10.14 years. The mean number of doses in our study was close to 5 days. Bacteriological investigation of bile reported that *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, and *Shigella sonnei* were the most frequently isolated pathogens. For patients with *Salmonella enterica*, the antibiotic course varied from 5 to 7 days, and they most frequently presented with a history of typhoid fever. Manan F, *et al.*<sup>11</sup> noted that the most common bacteria found was *Escherichia coli* 28 (22.22%) followed by *Klebsiella pneumoniae* 22 (17.46%), *Salmonella enterica* 16 (12.70%) and *Shigella sonnei* 8 (6.35%). The study also reported *Escherichia coli* to have high resistance to Amoxicillin in 17 (60.71%) patients followed by resistance to Ciprofloxacin in 12 (42.86%) patients whereas *Klebsiella pneumoniae* showed high sensitivity to Ciprofloxacin in 13 (72.22%) patients. Al Harbi M, *et al.*<sup>14</sup> noted that the most common organism isolated was *Escherichia coli* as 9 (28.1%) while *Klebsiella pneumoniae* was 2 (6.3%). He reported 88.8% sensitivity of *Escherichia coli* with Cefuroxime.

Moazeni-Bistgani M, *et al.*<sup>15</sup> reported that the most common isolated organism was *Escherichia coli*, i.e., 26% followed by *Salmonella enterica* 14% and *Klebsiella pneumoniae* 4%. Kaya M, *et al.*<sup>16</sup> reported that *Escherichia coli* (28.2%) was the most frequently encountered bacteria. Sahu MK, *et al.*<sup>17</sup> further stated in his study about the prevalence of *Escherichia coli* and *Klebsiella pneumoniae* as 34 (19.1%) and 7 (3.9%) respectively. Furthermore, he also claimed that the most effective antibiotics were sequentially Amikacin, Ceftriaxone, and Clindamycin as *Escherichia coli* was 92.3% susceptible to Amikacin, while it was nearly resistant to Erythromycin (90%). A study by Moazeni-Bistgani and Imani presents valuable information on the antimicrobial susceptibility patterns of bacteria isolated from bile samples of patients with cholelithiasis. The study shows that a majority of the bacteria isolates exhibited resistance to at least one antibiotic, indicating a high prevalence of antimicrobial resistance among these bacteria which is in line with our findings.<sup>15</sup>

Thapa SB, *et al.*<sup>18</sup> reported that *Escherichia coli* showed high sensitivity to Imipenem (100%) and least to ciprofloxacin (70%). Kaya M, *et al.*<sup>16</sup> also reported that most susceptible antibiotics were Imipenem

(79%), Ciprofloxacin (52%) and Cefotaxime (14%). Sahu MK, *et al*<sup>17</sup> noted that *Escherichia coli* showed sensitivity to Cefotaxime in 15.7% patients followed by Ciprofloxacin in 14.3% and Imipenem in 100% patients whereas *Klebsiella pneumoniae* showed sensitivity to Cefotaxime in 10% patients followed by Ciprofloxacin in 20% and Imipenem in 100% patients. Adequate antibiotics are necessary for patients who are operated for symptomatic cholelithiasis and its complications. It is of great importance to periodically assess the common patterns of resistance and sensitivity against common antibiotics to provide the optimum antibiotic therapy to hospitalized patients.<sup>19,20</sup> Noncompliance of antibiotic drugs and overuse of antibiotics are major contributing factors in increasing the resistance against bacteria in our population. 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

It is to be concluded that the most common bacteria of symptomatic cholelithiasis are *Escherichia coli* and *Klebsiella pneumoniae* followed by *Salmonella enterica* and *Shigella sonnei*. These bacteria showed an overall susceptibility to Imipenem. Additional studies are required to confirm our findings, probably with a larger sample size and with more parameters in multiple study centers in Pakistan are needed to confirm the findings of the present study.

## CONFLICT OF INTEREST

Authors declare no conflict of interest

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None

## AUTHOR CONTRIBUTION

**Kanwal Hameed:** Conception, analysis, interpretation of data and manuscript writing

**Asif Ali Amir Ali:** Conception, the acquisition, Analysis and interpretation of data and manuscript writing

**Sughra Parveen:** Data analysis, data Interpretation

**Imran Khan:** Revised critically for important intellectual content

**Jehangir Ali, Abdul Waheed:** Data Collection

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