



## BACTERIOLOGICAL SPECTRUM OF BILE CULTURE AND ITS ANTIBIOTIC SENSITIVITY PATTERN: A STUDY FROM A TERTIARY CARE CENTRE IN BIHAR

### Microbiology

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### ABSTRACT

Gallstones if present within the gall bladder or biliary tree is often complicated by cholangitis, which ranges from mild to severe life threatening conditions. This study was conducted with an aim to evaluate the microbiological profile of bile from gall bladder in patients undergoing cholecystectomy and to study their antibiotic sensitivity pattern. A total of 40 bile samples were cultured, out of which 20 samples (50%) were found to be culture positive. Most common organism isolated from the culture was *Klebsiella pneumoniae* (30%), followed by *Escherichia coli* (25%), *Pseudomonas spp.* (20%), *Acinetobacter spp.* (10%) and others. Almost all the isolates were sensitive to higher antibiotics like colistin and tigecycline, with varying degree of sensitivity against other antibiotics.

### KEYWORDS

Gallstones, Cholecystectomy, bile culture and sensitivity.

### INTRODUCTION

The bile within the bile ducts are normally considered to be sterile. Under conditions of normal bile flow, bacteria in the biliary system are of no clinical significance. Gallstones if present within the gallbladder or biliary tree, is associated with bacterial colonization of the bile. [1] In cases of absence of any stone, previous biliary interventions if done, is associated with high rates of bacteriobilia. [2,3] When there is bile duct obstruction, bacteria proliferates within the stagnant bile along with the increasing biliary pressure. This increased pressure presumably helps in translocation of bacteria into the circulation causing systemic infection. Biliary obstruction is often complicated by cholangitis, which ranges from mild to severe life threatening conditions and can also cause multiple organ dysfunction syndrome. [4,5] Enabling biliary drainage through endoscopic treatment and the use of antibiotics are mainstays of treatment in this condition. [5] Also it has been seen that starting prophylactic antibiotics before cholecystectomy has less incidences of post-operative infections. [4,6] The basis for choosing an empiric antibiotic regimen has several limitations. Data pertaining to the microbiological flora of the biliary tract are insufficient in literature. Also there are several methodological limitations like sample collection during surgical or percutaneous interventional procedures. Fewer studies have been performed regarding the bacterial spectrum of bile culture and their antibiotic sensitivity pattern. Also, there is no consensus opinion on the optimum initial antibiotic regimen which should be started in such cases. [7]

### AIMS AND OBJECTIVES

- To evaluate the microbiological profile of bile from gall bladder in patients undergoing cholecystectomy.
- To study the antibiotic sensitivity pattern of organisms isolated from biliary samples.

### MATERIAL AND METHODS

This retrospective record based study was carried out at Indira Gandhi Institute of Medical Sciences, Patna between January to December 2017, in the microbiology laboratory. The study was initiated after obtaining clearance from the Institutional Ethics Committee.

All Patients undergoing cholecystectomy for the complains of gallstone, were included in the study. Patient's demographic data like name, age, and sex were noted. Detailed history was taken with physical examination and investigations were done. Pediatric (<15 yrs

of age) and immunocompromised patients, septic patients with any known source, patients with history of ascending cholangitis, patients with preoperative diagnosis of empyema of gallbladder, patients who had undergone endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sphincterotomy within one week before surgery were excluded from the study.

The bile sample received in the microbiology laboratory was processed as per the standard protocols. Culture was performed on the Blood agar, Chocolate agar and MacConkey agar media and Antimicrobial sensitivity testing (AST) was performed by Kirby Bauer disc diffusion method. [8] For some antibiotics like Colistin and Tigecycline, AST was done using E-test method. [8] The isolates were identified using conventional biochemical reactions and the AST pattern was noted down.

Statistical analysis was done using SPSS version 24. Results were calculated as percentages and were presented in tabular form.

### RESULTS

A total number of 40 bile samples from equal number of patients undergoing cholecystectomy were received. Out of this, 20 samples (50%) were found to be culture positive. 6 patients were below 50 years of age, whereas 14 patients were above 50 years. Most common organism isolated from the culture was *Klebsiella pneumoniae* (30%), followed by *Escherichia coli* (25%), *Pseudomonas spp.* (20%) and *Acinetobacter spp.* (10%). Single isolates, each of *Citrobacter spp.*, *Enterobacter spp.* and *Proteus mirabilis* were also obtained from three different bile samples respectively (Table 1). The Antibiotic sensitivity pattern is described in Table 2. Almost all the isolates were sensitive to higher antibiotics like Colistin and Tigecycline, whereas varying degree of sensitivity was noted against other antibiotics. (Table 2)

**Table 1. Distribution Of Different Organisms In Positive Bile Cultures [n (%)]**

<i>Escherichia coli</i>	5 (25%)
<i>Klebsiella pneumoniae</i>	6 (30%)
<i>Pseudomonas spp.</i>	4 (20%)
<i>Acinetobacter spp.</i>	2 (10%)
<i>Citrobacter spp.</i>	1 (5%)
<i>Enterobacter spp.</i>	1 (5%)
<i>Proteus mirabilis</i>	1 (5%)

**Table 2. Sensitivity In Percentage Of Aerobic Bacterial Isolates From Bile**

Antibiotics	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas spp.</i>	<i>Acinetobacter spp.</i>	<i>Citrobacter spp.</i>	<i>Enterobacter spp.</i>	<i>Proteus mirabilis</i>
CIP	20	33	50	50	00	100	00
GEN	80	50	50	50	100	00	100
AK	100	50	75	50	100	100	100

AMC	00	17	25	50	00	00	100
CAZ	00	50	50	00	00	00	100
CFP	00	50	75	50	00	00	100
TOB	60	50	75	50	00	100	100
AZT	60	50	100	50	00	100	100
PI	NT	NT	50	NT	NT	NT	NT
PIT	60	50	100	50	00	100	100
CTX	NT	NT	50	NT	NT	NT	NT
CPD	NT	NT	00	NT	NT	NT	NT
IMP	100	83	50	50	00	100	100
TG	100	100	NT	100	100	100	100
CL	100	100	100	100	100	100	100

#### ABBREVIATION

CIP: Ciprofloxacin, GEN: Gentamicin, AK: Amikacin, AMC: Amoxycyclav, CAZ: Ceftazidime, CFP: Cefepime, TOB: Tobramycin, AZT: Aztreonam, PI: Piperacillin, PIT: Piperacillin-Tazobactam, CTX: Ceftriaxone, CPD: Cefpodoxime, IMP: Imipenem, TG: Tigecycline, CL: Colistin, NT: Not tested

#### DISCUSSION

All the bile samples from 40 patients undergoing cholecystectomy were included in the study, out of which 20 samples (50%) were culture positive. Similar results were obtained by Kaya M et al in their study on bile cultures from endoscopic retrograde cholangiography patients where the positivity was 50.5%. [9] Parikh PM et al found 24.36% culture positive bile and 75.64% culture negative bile. [4] In yet another study only around 32% of bile samples were positive, whereas 68% were sterile. [7] This variation may be due to more number of patients involved in these studies.

In all the 20 samples which were found positive in our study, only Gram negative organisms were grown in culture. Interestingly, not a single sample showed the growth of any Gram positive organism. In other studies, though Gram positive organisms were found, their numbers were very less. [4,5,7,9,10] However, a study done by Rupp C et al showed the predominance of Gram positive organisms (57%) among the 1491 bacterial isolates from a total of 1150 biliary samples collected through endoscopic retrograde cholangiography. [5] Again these differences may be attributed to the varying sample size and the techniques of biliary sampling.

The most common organism isolated in our study was *Klebsiella pneumoniae* (30%), but in other studies, *Escherichia coli* has been found to be the most common organism cultured from bile sample. [4,7,9-11] The percentage isolation of *E. coli* in our study was 25%, which was the second most common isolate. *Pseudomonas* was the third most common organism grown in culture in this study, which is in accordance with the study done by Shetty AS. [7] In few studies, *Enterobacter* was the third most commonly isolated Gram negative organism [5,11], but in our study, it was found only in a single bile sample. As far as the other isolates in our study like *Acinetobacter*, *Proteus* and *Citrobacter* are concerned, the scenario is more or less consistent with many other studies, where their rate of isolation is on the lower side. [5,7,9-11]

The antibiotic susceptibility testing was done in all cases as per the CLSI guidelines.[8] Almost all the isolates were sensitive to higher antibiotics like colistin and tigecycline, whereas varying degree of sensitivity was noted to other antibiotics. All the *E. coli* isolates were sensitive to imipenem, whereas the sensitivity exhibited by *Klebsiella* and *Pseudomonas* to imipenem were 83% and 50% respectively. Both ciprofloxacin and co-amoxycyclav are oral antibiotics and can be easily administered but strikingly, their sensitivity percentage in our study was found to be very less in almost all the isolates, which shows their rising resistance trend among organisms like *E. coli*, *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Citrobacter* and others. Cephalosporins like ceftazidime and cefepime were found to be ineffective against all the *E. coli* isolates whereas it showed decreased sensitivity against other organisms. This high resistance may be commonly related to inappropriate use of these antibiotics in this region. Amikacin, gentamicin, tobramycin, azithromycin and piperacillin-tazobactam have shown more than 50% sensitiveness towards almost all the isolated organisms. In many studies available in literature [4,5,7,9,11], the sensitiveness or the resistance of a particular antibiotic is shown as a whole, and not along with their respective organisms against which

they were tested. But in our study, we have tried to demonstrate the individual sensitivity of all organisms against all the antibiotics (Table 2), which would definitely help us in knowing the changing trend of antibiotic sensitivity pattern as regards to different individual bacteria.

#### CONCLUSION

In the patients of gallstone undergoing cholecystectomy, infections with gram negative organisms are common with *Klebsiella pneumoniae* dominating the list, followed by *Escherichia coli*, *Pseudomonas*, *Acinetobacter* and other organisms like *Citrobacter*, *Enterobacter* and *Proteus*. We recommend the primary use of antibiotics like amikacin, gentamicin tobramycin, azithromycin or piperacillin-tazobactam for the effective coverage of these gram negative organisms. Although the higher antibiotics seem to be highly effective against the organisms, the role of other simpler antibiotics cannot be completely denied. More similar type of studies needs to be conducted preferably with higher number of samples in order to get a complete picture of the varying bacteriological profile of these bile samples and their varying trends of antimicrobial sensitivity.

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