

Original Research Article

Clinico-microbiological analysis of bactibilia isolates in patients of cholecystectomy

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ABSTRACT

Background: The current study determined microbial flora in bile aspirates from patients undergoing cholecystectomy along with antibiotic susceptibility pattern and resistance mechanism viz. extended spectrum β -lactamase (ESBL).

Methods: The study included 110 such patients which were divided in three groups as: Group A that included acute cholecystitis with or without cholelithiasis, Group B included chronic cholecystitis with or without cholelithiasis and Group C included other hepatobiliary ailments requiring cholecystectomy viz. gallbladder carcinoma, acute emphysematous gall bladder and mucocele gall bladder. The bile was subjected to complete microbiological and histopathological examination. Antimicrobial susceptibility of the isolates was performed as per CLSI guidelines.

Results: Bacteria were recovered from 07 samples (20%) in Group A, 17 (48.57%) in Group B and 11 (31.43%) in Group C. The polymicrobial infection was seen in 04 (11.43%) patients. The most common organisms isolated were *Escherichia coli* 15 (38.46%), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* 8 (20.51% each). The majority of *Enterobacteriaceae* isolates were susceptible to piperacillin-tazobactam and carbapenems. The ESBL production was observed in 17 (43.58%) isolates.

Conclusions: Therefore, antimicrobial susceptibility of potential causative organisms, presence of resistant strains in bile, the severity of the cholecystitis, and the local susceptibility pattern must be taken into consideration while prescribing antibiotics. A protocol regarding the management of such cases should be formulated based on observations of similar studies.

Keywords: Acute and chronic cholecystitis, Bactibilia, ESBL, Gallbladder carcinoma

INTRODUCTION

Disorders of the gallbladder (GB) are a major health problem in developed as well as developing societies and usually associated with gallstones which have been labelled as most common biliary pathology. Symptomatic gallbladder disease can present in multiple ways commonest being acute cholecystitis. Microbial flora in GB disease has been detected in upto 80% of the patients who present with acute symptoms and in >25% patients

with chronic inflammation of GB. Although, gram negative preponderance has been seen in all GB disorders, polymicrobial infection have been found in tertiary care setup. Monomicrobial infection with gram-positive and anaerobic are uncommon causative agents. Viral and fungal agents are rare.¹

Gallbladder diseases in Indians are known to occur earlier, especially, in middle aged fat (obese) multiparous women. North India is the leading population in India

where gallbladder disease is seven times more common as compared to other parts of the country. The patients are treated repeatedly with ineffective multiple courses of antibiotics and the median period is nearly three years before they present for surgical procedures.²

In view of the repeated use of antibiotics, resistant virulent strains have been isolated in bile cultures from patients during cholecystectomy. This adds to the morbidity and in some cases mortality of patients with GB disease undergoing surgical procedures.³

In order to delineate this behavior of gallbladder disorders, a prospective study was planned where in current spectrum of organisms in bile aspirates obtained from patients during cholecystectomy and their pattern of antibiotic susceptibility in the settings of a tertiary care hospital has been determined.

METHODS

One-hundred and ten patients of biliary diseases were diagnosed and treated in the department of microbiology and surgery, Pt. B. D. Sharma PGIMS, Rohtak, Haryana, during the period of one year (2015-2016). For final analysis, the bile samples were divided in three groups as: Group A that included acute cholecystitis with or without cholelithiasis, Group B included chronic cholecystitis with or without cholelithiasis and Group C included other hepatobiliary ailments requiring cholecystectomy viz. gallbladder carcinoma, acute emphysematous gall bladder and mucocele gall bladder. The demographic profile and clinical examination of these patients were studied and noted in a proforma.

Routine examination of complete blood count, urine and serum biochemistry, and chest X-ray were done. An ultrasonogram of the upper abdomen was carried out in all patients to identify the site of obstruction, number of stones and condition of gallbladder. A CT-scan was also performed in patients with clinical suspicion of carcinoma where confirmation could not be done on ultrasonography. The open or laparoscopic cholecystectomy was performed as per patient condition. Bile aspirates were collected during cholecystectomy and sent to the microbiology laboratory in sterile syringe wrapped in a sterile gauze within an hour of collection at room temperature.

The bile was subjected to complete microbiological examination while resected gallbladder and stone were submitted for histopathological examination. All the samples were processed as per standard microbiological procedures which included direct microscopy using Gram staining and then directly inoculating onto various bacteriological media. These were also processed further in selective (selenite-F broth) and non-selective (brain heart infusion) broth. The isolates were identified by routine biochemical tests as well as by automated BD Phoenix system.

All the isolates were subjected to antibiotic susceptibility testing using Kirby-Bauer disc diffusion method done on Mueller Hinton agar plate by using the following antimicrobial agents, as per the CLSI guidelines:

For gram-negative bacteria

Ampicillin (10µg), Gentamicin (10µg), Amikacin (30µg), Amoxicillin-clavulanate (30µg), Cefepime (30µg), Piperacillin-tazobactam (100/10µg), Cefuroxime (30µg), Cefoxitin (30 µg), Ciprofloxacin (5µg), Imipenem (10µg), Meropenem (10µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), Cefotaxime (30µg), Chloramphenicol (30µg), Doxycycline (30µg), Ceftazidime (30µg), Aztreonam (30µg), Colistin (10µg), Polymyxin B (300units). For quality control of disc diffusion tests American Type Culture Collection (ATCC) control strains of *Escherichia coli* ATCC 25922 was used.

For gram-positive bacteria

Erythromycin (15µg), Penicillin (10Units), Cefoxitin (30µg), Trimethoprim-sulfamethoxazole (12.5/23.75µg), Linezolid (30µg), Doxycycline (30µg), Clindamycin (2µg), Vancomycin (30µg), Rifampin (5µg), Erythromycin (15µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ampicillin (10 units). For quality control of disc diffusion tests ATCC control strains of *Staphylococcus aureus* ATCC 25923 was used.

ESBL production in gram-negative organism was tested by double-disc synergy method as per CLSI guidelines. For quality control of ESBL confirmatory test by disc diffusion, control strains of *Klebsiella pneumonia* ATCC 700603 and *Escherichia coli* ATCC 25922 was used.

Statistical association was tested using Chi-square and Z-test for proportions. Statistical significance was considered when $p < 0.05$. Software used for analysis was SPSS 20.0.

RESULTS

The age range in the study population is from 17 to 70 years with mean age of 44.52 ± 12.56 years. Majority of the patients were female i.e. 85 (77.27%) and male constituted only 25 (22.72%). The ratio of F:M was 3.4:1. Group A consisted of 16 patients, Group B consisted of 81 patients and Group C consisted of 13 patients. Presence of at least one of the established risk factors were seen in majority 93 (84.54%) of the patients. All the patients in the study population had biliary colic presenting from variable period ranging from 3 days to 4 years. The commonest clinical presenting sign was tenderness with grade 1+ in 50 (45.45%), grade 2+ in 36 (32.72%) and grade 3+ in only 02 (1.80%) patients.

Table 1: Group wise distribution of the isolates in patients with bactibilia.

Bacterial isolate (n=39)*	Group A (n=07)	Group B (n=17)	Group C (n=11)
<i>Escherichia coli</i> (n=15)	03	08	04
<i>Klebsiella oxytoca</i> (n=8)	01	05	02
<i>Pseudomonas aeruginosa</i> (n=8)	01	03	04
<i>Citrobacter freundii</i> (n=2)	01	--	01
<i>Acinetobacter baumannii</i> (n=2)	--	01	01
<i>Enterobacter aerogenes</i> (n=2)	01	01	--
<i>Staphylococcus epidermidis</i> (n=2)	--	02	--
Total	07	20	12

*Four patients had polymicrobial infection, hence, total isolates are 39.

From a total of 110 patients, bacterial growth was seen in 35 (31.81%) patients. Majority of the patients with

bactibilia were in chronic cholecystitis group (B) i.e. 15 (42.86%) followed by other hepatobiliary ailments group (C) i.e. 11 (31.43%) and acute cholecystitis group (A) i.e. 09 (25.71%). Polymicrobial infection was seen in 04 (11.43%) patients.

The gram-negative preponderance was seen in all the three groups with *Escherichia coli* being most common in group A and B and *Pseudomonas aeruginosa* was isolated in majority of the patients in group C. The association of bacteriological profile with the groups was found to be statistically significant (p value=0.015) (Table 1).

Among the polymicrobial isolates majority were in Group B with 03 (75%) and only 01 (25%) seen in Group C whereas no polymicrobial infection was observed in Group A. The most common isolate seen in polymicrobial infection was *K oxytoca* (75%) followed by *E coli* and *S epidermidis*. The association of polymicrobial infection with the group wise distribution was found to be statistically non-significant (p value=0.142) (Table 2). The most effective drug in-vitro was carbapenems and piperacillin-tazobactam (Table 3).

Table 2: Group wise distribution of polymicrobial infection in patients with bactibilia (n=4).

Bacterial isolate	Group A (n=07)	Group B (n=17)	Group C (n=11)
<i>Escherichia coli</i> + <i>Klebsiella oxytoca</i>	--	01	01
<i>Klebsiella oxytoca</i> + <i>Staphylococcus epidermidis</i>	--	01	--
<i>Acinetobacter baumannii</i> + <i>Staphylococcus epidermidis</i>	--	01	--
Total	00	03	01

Table 3: Antibiotic susceptibility of isolates in the study population.

Bacterial isolate* (n=39)	AMP	AMC	AK	GEN	CPM	CAZ	AT	IMP	MRP	CIP	COT	PTZ
<i>Escherichia coli</i> (n=15)	3	5	6	5	6	3	2	12	10	6	8	8
<i>Klebsiella oxytoca</i> (n=8)	2	2	1	4	2	2	2	7	7	2	5	4
<i>Citrobacter freundii</i> (n=2)	1	0	0	1	0	1	2	1	2	0	2	2
<i>Enterobacter aerogenes</i> (n=2)	1	1	1	2	2	1	2	2	2	1	1	2
<i>Pseudomonas aeruginosa</i> (n=8)	-	-	0	2	4	0	4	4	5	3	-	2
<i>Acinetobacter baumannii</i> (n=2)	-	-	0	0	0	0	-	2	1	0	1	0

AMP=Ampicillin, AMC= Amoxicillin-clavulanate, AK= Amikacin, GEN=Gentamicin, CPM= Cefepime, CAZ= Ceftazidime, AT= Aztreonam, IMP= Imipenem, MRP= Meropenem, CIP= Ciprofloxacin, COT= Co-trimoxazole, PTZ= Piperacillin-tazobactam.

**Staphylococcus epidermidis* (n=2) sensitive to doxycycline, linezolid, clindamycin, gentamicin, co-trimoxazole and levofloxacin.

It was observed that out of 39 bacterial isolates, 84.61% were multidrug-resistant. None of the isolate was either

sensitive or resistant to all the drugs tested in-vitro (Table 4). Of the 37 gram-negative bacterial isolates, 17

(45.95%) were ESBL producers. The association of ESBL production with group wise distribution of isolates was found to be statistically significant (p=0.029) (Table 5). For Group A and B patients histopathological

evidence was consistent with the findings of acute and chronic cholecystitis respectively. For Group C patients, histopathological examination showed adenocarcinoma in 6 cases.

Table 4: Prevalence of drug resistance in bacteria isolated from patients of bactibilia in various groups.

Bacterial isolate	No. of isolates (n=39)	Resistant to all drugs	Resistant to ≥ three drugs	Sensitive to all drugs
<i>E. coli</i>	15	0	13	0
<i>K. oxytoca</i>	08	0	06	0
<i>C. freundii</i>	02	0	02	0
<i>E. aerogenes</i>	02	0	01	0
<i>P. aeruginosa</i>	08	0	07	0
<i>A. baumannii</i>	02	0	02	0
<i>S. epidermidis</i>	02	0	02	0
Total	39	0	33 (84.61%)	0

Table 5: Association of ESBL production in different study groups in gram-negative isolates.

Parameter	Group A (n=07)		Group B (n=17)		Group C (n=11)	
	With cholelithiasis	Without cholelithiasis	With cholelithiasis	Without cholelithiasis	With cholelithiasis	Without cholelithiasis
ESBL (n=17)	01	--	06	04	04	02
Percentage (%)	5.88	0	35.29	23.52	23.52	11.76
Total (%)	5.88		58.82		35.29	

Open cholecystectomy was performed in 73 cases (66.36%) and laparoscopic cholecystectomy was performed in 34 cases (30.91%). No disparity was observed between patients undergoing these two procedures as regards the morbidity associated with postoperative period.

DISCUSSION

Gallbladder disease, commonly manifests as gallstones. Patients often remain undiagnosed because cholelithiasis may not cause symptoms and often the patient presents with complications viz. acute and chronic cholecystitis. Gallbladder disease in Indians are known to occur earlier especially, in middle aged fat (obese), multiparous women. The mean age in the study population was 44.52±12.56 and the female:male ratio was 3.4:1. Similar findings have been observed in prior studies conducted in North India and Pakistan.^{4,5}

In the present study, 84.54% patient were found to have one or other of the established and identified five risk factors of the gall bladder disease. More than two factors were present in majority i.e. 54.54% whereas more than three factors were seen in 47.27% patients. The most common risk factor was female gender i.e. 77.27% followed by obesity i.e. 54.54%. Studying the risk factor profile for gall bladder disease in healthy individuals provide a unique opportunity to explore factors leading to

development of disease, long before a diagnosis of cholelithiasis is made.^{6,7}

Significant number of patients in Group B with chronic biliary diseases had culture positive bile (48.47%) as compared to patients in Group A with acute cholecystitis (20%). Present study co-relates with the study conducted in North Carolina on chronic cholecystitis patients.⁸ However, various studies across the globe have reported higher rates of isolation in acute cholecystitis patients as well.^{9,10} This higher rate of isolation among the patients of chronic cholecystitis (group B) in the present study may be because bacterial overgrowth in gallbladder deformed and fibrosed by repeated infections and healing becomes a sanctuary for bacteria to persist and grow especially when repeated ineffective doses of antibiotics are given to these patients during acute exacerbations.

Present study showed that preponderance of gram-negative infections in the biliary tract continue to be a major threat in management of the patients with gall bladder disease. *E. coli* was the most common pathogen isolated from bile aspirates (38.46%), followed by *K. oxytoca* and *P. aeruginosa* (20.10% each), *C. freundii*, *A. baumannii* and *E. aerogenes* (5.12% each). The significance of *E. coli* dominance is also supported by previous reports indicating a potential role for *E. coli*'s glucuronidase enzymatic activity in formation of calcium bilirubinate gall stone.^{4,9} The results of the present study

are in accordance with the previous studies by various authors.^{4,11} The association of monomicrobial and polymicrobial bacteriological profile was found to be statistically significant ($p=0.006$).

The association of polymicrobial infection with obstruction due to cholelithiasis or previous manipulation of the biliary tract has been widely reported by various authors.^{9,12,13} The polymicrobial infection in the present study was seen in 4 (11.43%) patients. It was mainly i.e. 75% associated with cholelithiasis with chronic inflammation. The importance lie in the fact that the stone may be acting as a source of infection with the bacteria trapped in the center, hence, in such cases it becomes imperative to remove stone prior to starting antibiotic therapy.¹⁴ Moreover, calculi is known to induce stasis, promoting chronic infection leading to increased turnover of primary bile acids to secondary bile acids, which causes constant irritation of the biliary tree and increased chances of infection.⁵ Acute suppurative cholangitis and cholangiohepatitis occur due to the presence of bacteria ascended from gut to lower bile duct due to various predisposing conditions viz. biliary stasis, cholelithiasis, stricture etc. and superinfection is very common in these patients as they undergo repeated episodes and seek medical care usually from local medical practitioners and receive repeated, suboptimal and ineffective doses of antibiotics leading to further worsening.^{3,5}

Anaerobes are infrequent causes of biliary infections and are usually associated with chronic inflammation due to other hepatobiliary ailments. Therefore, with this in mind, anaerobic culture was not performed in the current study which was primarily done on outdoor patients. In the current study, patients were initiated on broad-spectrum antibiotics to avoid postoperative anaerobic infection.⁵

Although surgical intervention remains the mainstay of therapy for gall bladder diseases and its complications, a period of hospital stay is required before elective or emergency cholecystectomy. The prior administration of antibiotic, gut colonization, emergency abdominal surgery like cholecystectomy, presence of gastrostomy and jejunostomy tube are well established and identified risk factors by various authors, for the development and spread of antibiotic resistant bacteria in regards to biliary infections.¹⁵ Numerous randomized studies have shown that there is no significant difference in postoperative complications in patients who received prophylactic antibiotics with that of the control study population.¹⁶

In the present series of patients, the majority of gram-negative isolates were susceptible to piperacillin-tazobactam and carbapenem. Increased rate of susceptibility of gram-negative isolated was seen for cotrimoxazole in the present study, which is not in concordance with the previous studies, depicting the change in antibiogram of bactibilia isolates. The gram-positive isolate were highly sensitive to linezolid,

clindamycin and doxycycline. The prevalence of multidrug resistant strains was 84.61% which is also higher than the previous studies conducted in this region.⁵ Prior studies observed excellent responses with quinolones for Gram-negative isolates and Vancomycin for Gram-positive isolates being preferred. With continued repeated use of these antibiotics for various ailments, may be the reason behind resurgence of resistance to organism.^{5,11}

The percentage rate of ESBL production in gram-negative isolates was 43.58% (17 out of 39 isolates). Notably it was seen only in strains of *E. coli* (11 i.e. 64.57%) and *K. oxytoca* (35.29%). The association of the patient's post-operative outcome with presence of ESBL producing bacteria in bile was noted and found to be statistically significant ($p=0.029$). Among the patients with ESBL producing bactibilia, majority i.e. 11 (94.11%) had an eventful post-operative course that include sustained fever, long hospital stays, septicemia and death. The overall mortality in the present study was 2.5%. Various randomized control studies have shown that there is significantly higher risk of developing sepsis postoperatively in patients with ESBL producing bactibilia.¹⁷

Hence, it is important to control the emergence and spread of ESBL for the reasons stated previously viz. the limited therapeutic alternatives, the increasingly compromised in-patient population, ever increasing polymicrobial infections with these resistant organisms and the potential for transfer of resistance to other pathogenic bacteria and development of further resistance e.g. to carbapenem. Furthermore, careless and widespread misuse of antimicrobial agents has increased the risk of antibiotic resistance. Inadequate doses of antibiotics, self-medication, counterfeit drugs, inadequate hospital measures promote the development of resistance in clinical isolates. In developing countries like ours, repeated, ineffective medication from local practitioners is a common practice especially in chronic diseases like chronic gall bladder diseases and this might probably be a major cause of antibiotic resistance. These patients carrying community acquired strains, on admission to hospital exchange the genetic information with the prevailing nosocomial isolates, resulting in emergence of multidrug resistant strains and polymicrobial infections.

CONCLUSION

It is therefore imperative that all patients undergoing cholecystectomy must have their bile aspirated during cholecystectomy and sent for microbiological examination and culture. It will guide the treating surgeons to use antibiotics appropriately which may improve the outcome of the patients and thereby may prevent morbid and mortality events. Thus, we recommend starting antibiotics selectively. However, being a small study, it is recommended that the data be extrapolated only after large population based trials

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