

Pattern of Bacterial Flora and Sensitivity in Bile Culture in Patient of Cholelithiasis Undergoing Laparoscopic Cholecystectomy

Nitin Kumar Chavan*, G. Sathish Kumar**

*Assistant Professor, Dept. of General Surgery, Sri Venkateshwara Medical college and Research Centre, Puducherry. **Assistant Professor, Dept. of General Surgery, IRT - Perundurai Medical College, Erode, Tamil Nadu.

Abstract

Introduction: Infective factor seems to be a major cause of formation of gallstones. Moynihan's aphorism that "gall stone is a tomb stone erected in the memory of the organism with in it" is true today [1]. Evidence in favour of infection includes isolation of *E. coli*, bacterium typhosum, streptococcus from the gallbladder bile and from the center of the gallstones.

Methodology: All patients who were subjected to laparoscopic cholecystectomy had their Gallbladder retrieved in a plastic endobag and bile collected in a culture bottle under strict aseptic precautions and transferred at room temperature in an hour to the laboratory for culture.

Results: The organisms grown from bile culture were *E-Coli* 45.5% (41/90), *Klebsiella* 27.7% (25/90), *Enterobacter* 8.8% (8/90), *Pseudomonas* 7.7% (7/90), *Staphyl-ococcus* 2.2% (2/90), Non-fermenting gram negative bacilli 2.2% (2/90), and *Citrobacter freundii* 1.1% (1/90).

Conclusion: Fluroquinolones (Levofloxacin/ Ofloxacin) may be used as first line drug for pre-operative prophylaxis in gallstone diseases undergoing surgery.

Keywords: Bacterial Flora; Bile Culture; Cholelithiasis.

Introduction

Biliary calculus disease is one of the most common disorder affecting the gastrointestinal tract and is important cause of morbidity. There has been marked rise in the incidence of gall stone disease in the west during the past century. In the UK, USA and Australia, the prevalence rate varies from 15-25%. In India, it is more common in the North India than in the South India. Similarly the incidence in Eastern India is higher than in Western India. Incidence of gallstones increases with the age. It is more common in female than male (M: F=1:4) and about 50% of patients are asymptomatic [1].

The pathogenesis of gallstones is multi factorial. It varies according to the type of gallstones. Primarily gallstones can be divided into two major groups. First is a pure gallstone contributing 10% of gallstones. Second is a mixed and combined gallstone which accounts for 90% of gallstones. Mixed gallstones frequently associated with cholecystitis. In about 1/2 of the cases bacteria can be cultured from the gallbladder bile [1].

Infective factor seems to be a major cause of formation of gallstones. Moynihan's aphorism that "gall stone is a tomb stone erected in the memory of the organism with in it" is true today [1]. Evidence in favour of infection includes isolation of *E. coli*, bacterium typhosum, streptococcus from the gallbladder bile and from the centre of the gallstones [1,2].

Pigment gallstones can be divided into two types. The first type of pigment gallstone is brown, earthy stone, which is said to occur principally in the orient. It consists mainly of calcium bilirubinate and calcium palmitate and is believed to develop as the result of infection and biliary stasis. The brown pigment stone

Corresponding Author: Nitin Kumar Chavan, Assistant Professor, Dept. of General Surgery, Sri Venkateshwara Medical College and Research Centre, Puducherry - 605102.
E-mail: nitinsurgery2016@yahoo.com

Received on 05.01.2017, Accepted on 10.01.2017

typically originate in the common bile duct in preference to gallbladder. The second type is the black pigment stone, which usually forms in the gallbladder, contains bilirubin polymers in addition to calcium bilirubinate [2].

Cetta has proposed that bacterial phospholipase may also provide the catalyst action for the precipitation of calcium palmitate, a component of primary bile duct stone. He suggested that bile infection precedes brown pigment stone formation in at least some patient, and most common organism is found to be E-Coli [3].

Methodology

All the patient presenting with gallstone disease admitted during study period were subjected to detailed history, appropriate clinical examination, laboratory and radiological investigations. After informed written consent, patients were subjected to laparoscopic cholecystectomy. Gallbladder retrieved in a plastic endobag and bile collected in a culture bottle under strict aseptic precautions. The collected bile was subjected to culture and sensitivity.

Inclusion Criteria

1. Symptomatic gallstone disease
2. Acute calculus cholecystitis
3. Chronic calculus cholecystitis

Exclusion Criteria

1. Acalculus cholecystitis
2. Gallstone with CBD stone
3. Patient unfit for surgery
4. Patient who refuse surgery

Sample Collection

All patients who were subjected to laparoscopic cholecystectomy had their Gallbladder retrieved in a plastic endobag and bile collected in a culture bottle

under strict aseptic precautions and transferred at room temperature in an hour to the laboratory for culture.

Method of Culture

In laboratory the bile samples were cultured in blood agar, McConkey agar, manitol salt agar and enrichment broth (thyoglycolate) and the plates were examined for bacterial growth at 48-72hrs. Bacterial growth was identified by gram staining, culture characteristics.

Antibiotic Sensitivity Test

The Kirby-Bauer disk diffusion test is used for determining antibiotic susceptibility. A pure culture of the test organism is inoculated on Mueller-Hinton agar. Paper disks impregnated with a standardized concentration of individual antibiotics are added to the plate which is then incubated at 35 degrees Celsius.

Results are read and interpreted at 18 hours of incubation. If the growth of a test organism is inhibited by the antibiotic on a disc, a zone of inhibition will appear. The zone of inhibition is a circular area surrounding an antibiotic disk in which the test organism does not grow. The diameter of the zone of inhibition is calibrated in millimeters and compared with the Interpretive Zone Standards published by the National Committee for Clinical Laboratory Standards. Results are reported as S for sensitive, I for intermediate, or R for resistant

Results

In our present study of 253 cases with gallbladder stone diseases, age group of 15-93 years were observed. In 253 cases female patients were 143 (56.5%) and male patients were 110(43.5%). More number of cases present in age group of 51-60 years.

In 253 bile samples for culture, 90(35.5%) were shown to have bacterial isolates. The organisms grown from bile culture were E-Coli 45.5%(41/90), Klebsiella 27.7%(25/90), Enterobacter 8.8%(8/90),

Table 1: Type of gallstone

Type of stone	Cases	
	No	%
Pigment stone	204	80.6
Cholesterol stone	40	15.8
Mixed stone	9	3.6
Total	253	100

Table 2: Bacteria isolated from Bile culture

Bile culture	Cases	
	No	%
No growth (Negative)	163	64.4
Growth(positive)	90	35.6
Citrobacterfreundii	1	1.1
E. Coli	41	45.5
Enterobacter	8	8.8
Klebsiella	25	27.7
Non fermenting gram negative bacilli	2	2.2
Proteus	4	4.4
Pseudomonas	7	7.7
Staphylococcus	2	2.2

Table 3: Sensitivity of various drugs

Drug	No.	%	Drug	No.	%
Ampicilin	13	14.5	Cefoperazone	37	41.1
Amoxicilin	10	11.1	Cafaclor	19	21
Amox - cla	11	12.2	Cafazolin	21	23.3
Imipinem	81	90	Cefdinir	42	46.6
Meropenem	83	92.2	Cefixime	19	21
Carbpenicillin	12	13.3	Ceftazidime	46	51.1
Piperacillin	45	50	Ceftizoxime	45	50
Ticarcillin	23	25.5	Cefotaxime	35	38.8
Co-tri.	29	32.2	Ceftriaxone	22	24.4
Doxycycline	60	66.6	Cefuroxime	22	24.4
Tetracycline	35	38.8	Cephalexin	9	10
Gentamycin	68	75.5	Cefpirome	27	30
Amikacin	78	86.6	Cefadroxil	2	2.2
Netilmycin	67	74.4	Cefepime	43	47.7
Tobramycin	64	71	Vancomycin	2	2.2
Ciprofloxacin	43	47.7	Methicillin	2	2.2
Gatifloxacin	69	76.6	Penicillin	2	2.2
Lomefloxacin	32	35.5	Roxithromycin	2	2.2
Levofloxacin	67	74.4	Clarithromycin	2	2.2
Norfloxacin	30	33.3	Azithromycin	2	2.2
Ofloxacin	57	63.3	Erythromycin	2	2.2
Sparfloxacin	49	54.4	Clindamycin	2	2.2
Moxifloxacin	54	60	Linazolid	2	2.2
Nalidixic acid	2	2.2	Teicoplanin	2	2.2

Pseudomonas 7.7%(7/90), Staphylococcus 2.2%(2/90), Non-fermenting gram negative bacilli 2.2%(2/90), and Citrobacter freundii 1.1%(1/90).

Overall antibiotic sensitivity for bacterial growth was highest for carbepenem group of drugs (90-93%), followed by aminoglycosides (66-87%), fluoroquinolones (54-76%), tetracyclines (40-66%), cephalosporins (20-51%), and penicillin (10-50%) group of drugs.

In 253 cases of cholelithiasis pigment gallstone found in 204 cases (80%), cholesterol stone were found in 40 cases (16%) and mixed stone in 9 cases (4%).

In 253 cases, Bile culture growth found in 90 cases (35.6%). Most common organism was found E-Coli 41 cases (45.5%), followed by Klebsiella 25 cases (27.7%), Enterobacter 8 cases (8.8%), Pseudomonas 7 cases (7.7%), Proteus 4cases (4.4%), Non fermenting gram negative bacilli and staphylococcus 2 cases each (2.2%) and Citrobacter 1 case (1.1%).

E.Coli shows highest sensitivity for carbepenem group of drugs (87-90%), followed by aminoglycosides(70-83%), fluoroquinolones (54-74%), tetracyclines (40-59%), cephalosporins (51-52%), and penicillin (40%) group of drugs.

Klebsiella, shows highest for carbepenem group of drugs (100%), followed by amino glycosides (80-92%), fluoroquinolones (60-92%), cephalosporins (44-75%), tetracyclines (36-80%), and penicillin (45%) group of drugs.

Enterobacter, shows highest for amino glycosides (60-100%), followed by fluoroquinolones (50-100%), carbepenem group of drugs (75-88%), tetracyclines (50-75%), cephalosporins (12-75%), and penicillin (50%) group of drugs.

Pseudomonas, shows highest for carbepenem group of drugs (85-86%), followed by amino glycosides (57-72%), cephalosporins (28-72%), fluoroquinolones

(15-72%), penicillin (72%), tetracyclines (28-43%), and group of drugs.

Overall antibiotic sensitivity for bacterial growth was highest for carbapenem group of drugs (90-93%), followed by aminoglycosides (66-87%), fluoroquinolones (54-76%), tetracyclines (40-66%), cephalosporins (20-51%), and penicillin (10-50%) group of drugs.

Discussion

In the majority of publications, 25-35% patients undergoing biliary surgery were found to harbour bacteria in the bile. In his study Mr. R.G. Willis of Park hospital, Manchester [4], bile was collected from 76 patient and 9 cases were positive for culture. Culture media used for bile samples were blood agar and McConkey agar without salt, Desoxycholate citrate agar, cooked meat media. Common organism isolated from bile in our study was E.coli as was in R.G. Willis and Lawson study.

Lygia Stewart and J. Macleod Grifiss studied 215 patients with symptomatic gall stone disease, 48 patients (22.3%) had bile culture positivity [5]. R.G. Willis study of 76 patients with symptomatic gallstone disease, 9 patients (11.8%) had bile culture positivity [4]. In our study of 253 patients with gallstone diseases, 90 patients (35.6%) had bile culture positivity.

Howards and Kaufman [6] from Department of Surgery, the John Hopkins Medical Institution studied 65 patients undergoing cholecystectomy for cholelithiasis, bile was cultured in different type of stones Gall bladder stones in 65 patients were identified as cholesterol gallstones in 46 patients (71%), pigment gallstones in 19 (29%) patients. Bile culture was positive in 4 patients in cholesterol gallstones, 7 cases in pigment gallstones. They conclude that black and brown pigment gallstones have different pathogenic mechanisms and that bacterial infection is important only in the formation of brown pigment stones.

In our series, 204 patients had pigment stone, 40 patients had cholesterol stone and 9 patients had mixed stone. Bile culture was positive in 73, 13 and 4 cases respectively with percentage of bile infection 35.8%, 32.5% and 44% respectively. Antibiotic sensitivity pattern was studied for all bacterial isolates, which showed highest sensitivity to carbapenem, fluoroquinolones, amino glycosides, and cephalosporins group of drugs. They concluded that bacterial isolates were found in pigment stone containing bile. Non lithogenic bile revealed no bacteria, showing no association between gallstone

formation and presence of bacteria in bile. Antibiotic sensitivity pattern of isolates organisms were similar irrespective of the type of stone. A prospective study on biliary bacteriology in calculi diseases of gallbladder and the role of common newer antibiotic was conducted in the department of surgery, Acharyashri Chander College of medical sciences and hospital Sidhra, Jammu. In their study of 150 cases of cholelithiasis bile culture was done, bacterial isolates from bile was found in 26 cases, of which most common growth was E-Coli 53.84% (14/26), followed by Pseudomonas 26.92% (7/26) and Staphylococcus aureus 19.23(5/26) [7].

Antibiotic sensitivity pattern was tested against cephalosporins group of drugs (cefuroxime, cefoperazone and cefepime) and its was found that sensitivity to 3rd and 4th generation was higher as compared to 2nd generation in acute as well as chronic cholecystitis.

Conclusion

- Most common organism isolated from bile culture was E-Coli.
- Overall highest antibiotic sensitivity of bacterial isolates was found with carbapenem group of drugs, followed by amino glycosides, fluoroquinolones, tetracycline, cephalosporins and penicillin group of drugs.

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