

Frequency and Type of Organisms in Gallstone Culture

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ABSTRACT

Background: Spillage of stones in peritoneal cavity is quite common in patients undergoing laparoscopic cholecystectomy. Lost stones may cause wide range of complications postoperatively which reflects the infective potential of these stones.

Objective: The aim of this study was to find out the frequency of viable bacteria in stones retrieved from the gallbladder after laparoscopic cholecystectomy & subjecting the stones for culture.

Design: Cross-sectional study.

Place & Duration: This study was conducted at Surgical Unit 4 of Civil Hospital Karachi, from July 2013 till March 2014.

Materials & Methods: 80 cases of cholelithiasis, selected & operated by laparoscopic cholecystectomy were included. During cholecystectomy, stones were collected, intact stones were sent in two separate containers from each subject, for microbiological examination.

Results: This study included 80 patients, 58 patients (72.5%) were female & 22 patients (27.5%) were male. Out of these 80 patients, 27 patients (33.75%) had positive stone culture, out of these 27 patients, 16 patients (20%) with positive core culture, 11 patients (13.75%) had positive surface culture. The commonest organism was E. coli in 13 patients (16.3%) followed by Pseudomonas in 3 patients (3.8%), Klebsiella in 3 patients (3.80%), Staphylococcus aureus in 2 patients (2.5%) & Bacteroides fragilis in 1 patient (1.3%). No salmonella species were isolated. All organisms were tested for sensitivity against quinolones, penicillins, cephalosporins, aminoglycosides & carbapenems.

Conclusion: This study proves the infective potential of gallstones, as they contain viable bacteria, which may cause post operative complications, therefore all spilled stones during surgery should be removed & peritoneal cavity should be meticulously cleared.

Keywords: Gallstones, laparoscopic cholecystectomy, core culture, surface culture, viable bacteria.

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INTRODUCTION

Laparoscopic cholecystectomy is the gold standard treatment for symptomatic gallstone disease, during surgery spillage of stones occurs in 6-40% of the procedures performed, while 13-30% of such operations results in lost stones^{1,2}. The complications from retained gallstones due to spillage in the peritoneal cavity can range from abscess formation to sepsis³⁻¹⁰, which suggests their infective potential. These complications are independent of contamination with bile¹¹,

furthermore bacteria have been demonstrated in core of gallstones through electron microscopy and molecular genetic techniques but does not prove that bacteria found in the cores of gallstones are live and capable of multiplying, and thus a potential cause of infective complications. Gallstone culture is the suitable method for demonstrating viable bacteria walled up within the calculus (core of gallstones), in patients undergoing cholecystectomy.

The aim of this study was to find out the organisms by harvesting the stones for culture, to confirm the viability of the organisms & finding out its pathogenicity & sensitivity to antibiotics. The resultant data may prove beneficial for pre- and post operative variables such as the use of prophylactic antibiotics, wound sepsis, local infection control protocols & hospital stay.

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Inclusion Criteria :

Patients with symptomatic gallstone disease.

Exclusion Criteria :

Patients with acute cholecystitis, cholangitis, jaundice, dilated CBD on ultrasonography, choledocholithiasis, any preoperative endoscopic intervention, perioperative findings of empyema of gallbladder, fistula, gangrene or iatrogenic perforation of gallbladder during procedure, patients who underwent open cholecystectomy, or converted from laparoscopic to open procedure. Patients with positive viral markers, diabetics, smokers were excluded.

METHODS

80 patients included in the study had symptomatic gallstone disease. Age range was 22-70 years. Workup included a preoperative ultrasound. laboratory studies, such as complete blood count, liver function tests, serum electrolytes, viral markers, chest radiographs, & cardiac function tests if indicated by the anaesthesia fitness guidelines. Procedures were carried out after written & informed consent. All patients underwent laparoscopic cholecystectomy by conventional method, gallbladder was removed from peritoneal cavity within a glove and opened, intact stones were picked up and transferred in two separate sterile containers labeled "A" & "B", containing normal saline and transported to laboratory for culture. In laboratory the intact stones from container "B" were pretreated with 70% alcohol for 15 minutes, stones were bisected & core was removed & subjected for gram positive and gram negative aerobic & anaerobic culture, while stones from container "A" were subjected for surface culture without pretreatment with alcohol. This study focused on stone culture regardless of postoperative outcome. Data was analysed on Spss v.20 for frequency.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

Table 1: Isolates of Gallstone culture with their sensitivities (Organisms were labeled sensitive ; S or resistant; R, only when >50% of the specific isolates belonged to that group, as there was presence of variable sensitivity & resistance in same organisms cultured from different subjects)

Organism	Frequency	"A" surface sample	"B" core sample	Quinolones	Penicillins	Aminoglycosides	Cephalosporins	Carbapenems
E.coli	13	3	10	S	S	S	S	S
Pseudomonas	3	2	1	S	R	-	R	-
Klebsiella	3	1	2	S	S	S	S	S
Staph.aureus	2	0	2	S	R	S	-	S
Bacteroides	1	0	1	-	R	S	S	-
Mixed Colony	5	5	0	-	-	-	-	-

RESULTS

This study included 80 patients, 58 patients (72.5%) were female & 22 patients (27.5%) were male. Out of these 80 patients, 27 patients (33.75%) had positive stone culture, out of these 27 patients, 16 patients (20%) with positive core culture, 11 patients (13.75%) had positive surface culture. The commonest organism was E.coli in 13 patients (16.3%) followed by Pseudomonas in 3 patients (3.8%), Klebsiella in 3 patients (3.80%), Staphylococcus aureus in 2 patients (2.5%) & Bacteroides fragilis in 1 patient (1.3%) & a mixed colony (containing two or more types of organisms) in 5 patients (6.25%). No salmonella species were isolated. All organisms were tested for sensitivity against quinolones, penicillins, cephalosporins, aminoglycosides & carbapenems. Refer to tables I through IV.

Table 2: Frequency of gender

	Frequency	Percent	Valid Percent	Cumulative Percent
Male	22	27.5	27.5	27.5
Female	58	72.5	72.5	100.0
Total	80	100.0	100.0	

Table 3: Type & frequency of flora in isolates

	Frequency	Percent	Valid Percent	Cumulative Percent
E.coli	13	16.3	16.3	16.3
Pseudomonas	3	3.8	3.8	20.0
Klebsiella	3	3.8	3.8	23.8
Staph.aureus	2	2.5	2.5	26.3
Bacteroides	1	1.3	1.3	27.5
Mixed	5	6.3	6.3	33.8
No Growth	53	66.3	66.3	100.0
Total	80	100.0	100.0	

Table 4: Sampling techniques. (Note that frequency of cultures showing no growth in the previous table corresponds with the frequency of equivocal label in sampling techniques as there was no growth on either of the samples in that particular specimen).

	Frequency	Percent	Valid Percent	Cumulative Percent
Surface Sample	11	13.8	13.8	13.8
Core sample	16	20.0	20.0	33.8
Equivocal	53	66.3	66.3	100.0
Total	80	100.0	100.0	

DISCUSSION

Bacteria inside the gallbladder have long been known thought to be Dead. "Gallstones are tombstones created in the memory of dead bacteria", Moynihans aphorism¹². Complications produced by spilled gallstones during laparoscopic cholecystectomy reveals their infective potential, although bile culture can demonstrate the infective potential of bacteria colonizing the gallbladder, it yields lower positive culture rate in patients with gallstones than stone culture, however a negative bile culture does not exclude the presence of bacterial biofilms on stones¹³⁻¹⁵. Moreover bile culture does not prove that bacteriobilia itself is the cause or effect of cholelithiasis. Our study reflects that stone culture is the only method to confirm the viability of the bacteria in gallstone calculus.

Enteric organisms have often been suspected of causing cholelithiasis¹⁶, and intestinal flora have frequently been recovered following interventions on the biliary tree. In our study stone culture revealed positive culture in 27 patients (33%), out of which 16 patients (20%) had positive core culture while 11 patients (13.8%) had positive surface culture, studies have reported upto 81% positive core culture¹⁷. The incidence of bile and gallstone infection varies from area to area in the world¹⁸. In this study the commonest enteric organism isolated was E.coli 13 patients (16.3%). The incidence of E.coli of about 15% has been reported in previous studies¹⁹, which is almost equal to our study. No salmonella organism was recovered from stones in our study. Reported incidence of Salmonella in other studies is (1.5%)²¹. Among the non enteric organisms in this study Pseudomonas & Staph. aureus were positive in 3 (3.8%) & 2 (2.5%) patients respectively. Mixed organisms were seen in 5 (6.3%) patients.

Studies have shown patients undergoing laparoscopic cholecystectomy for cholelithiasis had positive carcinoma gallbladder in 14% & positive stone culture has been reported to be 77%, from these patients^{20,21}. It is of interest that only enteric pathogens were recovered from the stones of patients with carcinoma of gallbladder. It is possible that chronic colonisation of the gallbladder with gallstones harbouring enteric bacteria may predispose to gallbladder carcinoma, but further studies are required before definitive conclusions can be drawn in this regards. So the prior knowledge about the type & sensitivity of the organisms can prove beneficial, should any complication related to gallstone spillage arise, not to mention the possibility of benefit from such data in other studies concerning prophylactic antibiotic administration & regulating local infection control policies.

CONCLUSION:

This study proves the infective potential of gallstones, as they contain viable bacteria, which may cause post operative complications, therefore all spilled stones during surgery should be removed & peritoneal cavity cleared meticulously.

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