The Culture Site of the Gallbladder Affects Recovery of Bacteria in Symptomatic Cholelithiasis

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Abstract

Aim. Traditional methods for bile culturing may miss a large number of underlying bacterial infections that could lead to acute or chronic cholecystitis. **Aim**: to evaluate possible differences regarding the site of material collection and thus to detect the most suitable sample site for gallbladder culture. **Methods.** A cohort of 137 patients with symptomatic cholelithiasis was enrolled. After surgical excision of the gallbladder, bile cultures were separately performed from fundus, body and neck. Identification of bacteria as well as computation of mean bacterial concentrations were performed with standard microbiological techniques. Wilcoxon's paired and Chi-square tests were used for comparison between continuous and discrete parameters, respectively. **Results.** Thirty-one patients (22.6%) demonstrated at least one positive culture sample. Positivity was 31/31 (100.0%) in neck samples, 20/31 (64.5%) in body and 13/31 (41.9%) in fundus samples (P<0.001). The microorganisms identified were *Escherichia coli* (14 cases) and *Enterococcus faecalis* (10 cases), followed by *Staphylococcus aureus* (3 cases), *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Enterobacter aerogenes* and *Enterobacter cloacae* (1 case each). Mean bacterial concentrations in positive samples derived from the neck (272.2 ± 187.5) were higher (P<0.01) when compared to those derived from both the body (38.2 ± 28.7) and the fundus (12.5 ± 11.3). Mean bacterial concentrations in positive samples derived from the body were higher (P<0.01) than those derived from the fundus. **Conclusion**. The neck of the gallbladder hosts the biggest bacterial load in comparison with the body and the fundus. This difference might be attributed to the presence of Rokitansky-Aschoff sinuses, which is the main histological characteristic of the region.

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Key words

Cholelithiasis – bacterial infection – bile.

Introduction

Chronic cholecystitis is a commonly encountered gallbladder disease, characterized by inflammation due to bacterial infection, which has been demonstrated to be strongly associated with the presence of gallstones [1]. Documented risk factors for gallstone disease include obesity, rapid weight loss, high dietary intake of fat, multiple deliveries and congenital hemolytic anemias such as sickle cell disease [2]. In Western countries, gallstones are mainly composed by cholesterol and bacteria have a minor role in their pathogenesis. Bacterial infections of the bile duct might play a critical role in the development of brown gallstones (formed mainly from bilirubin), while stones may format a background for bacterial development in some cases [3-8]. Prompt administration of the appropriate antibiotics is crucial in the management of biliary tract infection and antimicrobial treatment is commonly administered pre- or peri-operatively and often inhibits the bacterial growth. Moreover, it is suggested that the recovery of the bacteria in bile cultures is affected by toxicity of bile salts [9-12]. Thus, traditional culturing methods of bile might miss a large number of underlying bacterial infections that could lead to acute or chronic cholecystitis.

In the present study, cultures were collected from three different anatomical parts of the gallbladder mucosa postoperatively from patients cholecystectomized due to symptomatic cholelithiasis. The aim of the study was to evaluate possible differences in the recovery of bacteria and thus to detect the most suitable site for sampling bile for culture.

Material and Methods

A cohort of 137 patients who were hospitalized for symptomatic cholelithiasis and underwent cholecystectomy in our surgical department, were prospectively enrolled in the study. Exclusion criteria were evidence of Systemic

Inflammatory Response Syndrome (SIRS) or sepsis, presence of malignant disease, immunodefficiency and prior use of antibiotics up to 3 months, except for perioperative chemoprophylaxis. All patients were asked to give their informed consent. The study was in agreement with the Declaration of Helsinki and was approved by the relevant Ethics Committee.

All 137 gallbladders were microbiologically tested after open or laparoscopic cholecystectomy, for which antimicrobial chemoprophylaxis with cefoxitin (1g IV) had been administered perioperatively at the induction of anesthesia. Three tissue samples were obtained immediately after the operation from each gallbladder. The first sample was prelevated from the mucosa of the fundus, the second from the mucosa of the body and the last one from the mucosa of the neck.

The removed gallbladder was incised and cultures were obtained from several parts as described. The sample was inoculated into agar plates within the next half hour.

Samples were collected by applying a sterile calcium alginate swab for 30 sec and swabs were directly inoculated onto the agar plates, which were as follows: Columbia blood agar, anaerobic blood agar, chocolate agar, staphstrep agar and McConkey agar. Plates were incubated under aerobic, microaerobic and anaerobic conditions and the microbial count of the bacteria was estimated. Bacterial identification was based primarily on the macroscopic and microscopic morphology. Colonies showing macroscopically morphological differences were subcultured and identified by the API 20 E and 20 NE systems (bioMérieux, La Balme Les Grottes, France) as well as by testing for various properties using key tests prepared in-house [13].

The statistical analysis was performed by the application of Chi-square test (for positivity of cultures) and Wilcoxon's paired test (for mean bacterial concentrations, as their number was <40 and their distribution presented deviation from normality). A P value of <0.05 was considered significant.

Results

Data of the isolated bacteria in relation with their sample site of gallbladder are presented in Table 1. Thirty-one out of 137 cases (22.6%) demonstrated at least one positive culture sample. The remaining 106 gallbladders were negative in all three samples. Positivity was 31/31 (100.0%) in neck samples, 20/31 (64.5%) in body samples and 13/31 (41.9%) in fundus samples (P<0.001). The commonest microorganisms identified were *Escherichia coli* and *Enterococcus faecalis*, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Enterobacter aerogenes* and *Enterobacter cloacae*. Polymicrobial infection was not detected in any sample.

The mean bacterial concentrations (colony forming units, CFU ± standard deviation) of the positive samples for the neck, the body and the fundus were 272.2 ± 187.5, 38.2 ± 28.7, and 12.5 ± 11.3, respectively. The prevalence of bacteria differed significantly (P <0.01) among the three sampling sites, being highest in specimens from the mucosa of the neck. Higher numbers of isolates were yielded from the neck than from the body or fundus specimens. Moreover, mean bacterial concentrations in positive samples derived from the body were higher than those derived from the fundus. All the above mentioned differences concerning mean bacterial concentrations have been calculated using Wilcoxon's paired test and yielded to P<0.01.

Table I. Isolated bacteria in relation with the sampling site of gallbladder

	Sampling site		
Isolated bacterial species	Fundus	Body	Neck
Escherichia coli	7	9	14
Enterococcus faecalis	2	7	10
Staphylococcus aureus	1	1	3
Pseudomonas aeruginosa	1	1	1
Enterococcus faecium	0	0	1
Enterobacter aerogenes	1	1	1
Enterobacter cloacae	1	1	1
Total positive samples	**13**	**20**	**31**

No statistical correlation between infection of the gallbladder with a specific microorganism and the number of positive samples was established.

Discussion

In the present study, we demonstrated that gallbladders excised from patients with symptomatic cholelithiasis, even while lacking any evidence of inflammation, might host bacteria in over 20% of cases. Moreover, the most usual, if not compulsory, site of bacterial growth in these cases is the neck of the gallbladder. These findings are of significant interest as the pathophysiology of cholelithiasis has not been entirely clarified yet and many queries are raised concerning the role of the gallbladder flora in the evolution of cholelithiasis in otherwise healthy subjects [2, 4].

The pathophysiological role of bacteria in the formation of gallstones was proposed long ago. Bacteria are often found in high concentrations in brown pigment and less so in cholesterol gallstones. Although it is intriguing to hypothesize that cholesterol stone formation is non-bacterial in nature and essentially different from the pathogenesis of "infectious" brown pigment gallstones, it is more likely that significant overlap exists between the two processes. This is in keeping with the fact that most gallstones are mixed in nature [4].

Using molecular-genetic methods, bacteria can be found in the most pure cholesterol gallstones (i.e. those containing more than 90% cholesterol). An interesting study [5] has shown that DNA sequencing of Polymerase Chain Reaction (PCR) products is a useful technique to detect fastidious pathogens, which are difficult to culture, and found that gram-positive cocci are associated with the formation of

pure cholesterol stones.

Apart from molecular techniques, many studies have used the classical methods of obtaining cultures to identify the microflora in the gallbladder of patients undergoing laparoscopic cholecystectomy for gallstones. Al Harbi et al [6] obtained bile samples from 112 patients undergoing elective laparoscopic cholecystectomy for gallstones and inoculated them directly into aerobic and anaerobic blood culture bottles in the operating theatre. Twenty-eight of these bile cultures (25%) were positive, four of which contained more than one organism. Similarly to our study, the most common organisms isolated were *E. coli* (9 cases; 28.1%), *E. faecalis* (5 cases; 15.6%) and *P. aeruginosa* (3 cases; 9.4%). In another study, Csendes et al [7] studied the number of bacterial colonies and the number of pyocytes per ml of choledochal bile. One hundred patients with symptomatic gallstones, 42 patients with common bile duct stones without cholangitis, 24 patients with common duct stones and acute cholangitis, and 42 controls were included in the study. The investigators detected no bacteria in the gallbladder bile of the controls. Only 3% of patients with gallstones had more than 105 colonies per ml; this number increased to 36% in patients with common duct stones without cholangitis and to 84% among patients with acute cholangitis ($p<0.001$). Polybacterial flora was more prevalent among patients with acute cholangitis and anaerobic bacteria were again not seen in patients with gallstones, in consistency with Al Harbi et al data [6]. As patients with acute cholangitis had significantly more pyocytes present in the choledochal bile, the authors concluded that there might be a direct correlation between the number of colonies present in the bile and the severity of biliary tract disease [7].

Lotveit et al [8] studied the role of juxta-papillary duodenal diverticula in gallbladder flora in patients with chronic cholecystitis and concrements in the gallbladder, but no biliary obstruction. A significantly higher incidence of positive cultures was found in patients with diverticula than in the control group. The positive samples contained bacterial species belonging to the intestinal flora. Thus, the authors proposed an ascending route of infection and assumed that bacteria might play a role in the formation of gallstones, so often present in patients with juxta-papillary duodenal diverticula.

Despite the suspected crucial role of bacterial pathogens, the natural history of the gallstones development is unknown. The development of gallstones (nucleation, assembly of microcalculi, growth, remodeling) includes the interaction of both bacterial and non-bacterial mechanisms, working discontinuously over years and decades and shaping the structural individuality of each stone. At cholecystectomy, the gallstone removed from the patient represents the end product of a long pathogenetic process. Although our understanding of the exact temporal contribution of bacteria in lithogenesis is incomplete, it is important for the clinician to realize that most gallstones are colonized by a bacterial biofilm, even though the bile culture may be negative [4].

Our results, showing that the mean bacterial concentrations in positive samples derived from the neck were higher when compared to those derived from both the body and the fundus raise queries about how the specific conditions in each anatomic part of the gallbladder may influence the flora. It is known that gallbladder mucosa consists of a simple columnar epithelium and a lamina propria of connective tissue, arranged in rugae. The middle muscular layer of the wall consists of smooth muscle fibers (muscularis) while the outer layer is the visceral peritoneum. The structure of the cystic duct is analogous to that of the bile tract (mucosa, smooth muscle fibers and elastic fibers). At the level of the neck, an important histological variant should be mentioned. The mucosa of gallbladder creates small tubulo-alveolar mucoid glands by intraflexion, which are present only in the neck. Moreover, it produces saccular invaginations, which can extend and perforate the muscular layer of the wall creating large saccular dilatations (sinuses of Rokitansky-Aschoff) as a consequence of inflammation [2]. These sinuses communicate directly with the lumen of the gallbladder. They are covered by the typical columnar epithelium and are often detected in diseases of the gallbladder related with impaired immune response, as well as in gallbladder carcinoma [14-16]. Their higher incidence in inflamed gallbladders raises the possibility that preexisting injury to the wall may predispose to their development.

It is also of interest that histological evaluation of inflammatory lesions of the gallbladder shows a preference of adhesions to the neck epithelium of mucosa, independently of the acute of chronic type of inflammation [17, 18]. The presence of bacteria in a clinically normal gallbladder with cholelithiasis has been demonstrated by Sakurai et al. who examined normal and inflamed gallbladders under electron microscopy. The authors demonstrated the existence of bacteria in the bile of a clinically normal organ. Another interesting point of the present study, compared to similar previous studies, is the incidence of the bacteria. Sakurai et al suggested a significantly decreased incidence of bacteria recovery in asymptomatic cholelithiasis [18].

To **conclude**, our results comply with previous studies regarding the presence of bacterial pathogens in the gallbladder of patients with symptomatic cholelithiasis, thus further enhancing their potent pathogenetic implication in the formation of gallstones. As far as the increased bacterial load detected in samples collected from the neck of the gallbladders from patients with symptomatic cholelithiasis without clinical evidence of acute or chronic inflammation, we further hypothetize that this difference could be attributed to the Rokitansky–Aschoff sinuses. This hypothesis, along with its pathophysiological consequences, needs further investigation through more targeted studies, as it has evident implications concerning the methodology by which culture material is obtained in these cases.

Conflicts of interest

None to declare.

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