Micro-organisms in the bile

A preventable cause of sepsis after biliary surgery

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Summary
The bile is infected in 31% of patients undergoing an operation for biliary disease and these patients have a significantly greater risk of developing wound sepsis and septicaemia than patients with sterile bile. Prophylactic antibiotics which achieve satisfactory serum rather than high bile levels have been shown to reduce the morbidity of biliary operation. However, only patients with infected bile benefit from prophylactic chemotherapy. Patients with infected bile can be satisfactorily identified by preoperative duodenal aspiration, operative Gram staining of bile, or clinical presentation. The high-risk patients requiring preoperative antibiotic cover include anyone over 70 years of age, jaundiced patients, those requiring emergency operation, patients with recent rigors, anyone having had previous biliary operations, and patients known to have choledocholithiasis.

Introduction
In this study the relationship between biliary infection and postoperative sepsis has been investigated. John Hunter[1] was aware of inflammatory conditions of the gallbladder, but he did not know that bacteria are frequently present in the obstructed biliary tract. It was not until 50 years after Pasteur's discoveries that Welch described the presence of bacteria in the bile and also in the centre of gallstones[2]. Current observations record a 30–50% incidence of bacteria in the bile during biliary operations[3, 4]. Biliary infection is much more common in jaundiced patients[5, 6] and in patients requiring emergency operation[7].

Operations on the biliary tract are associated with a 20% incidence of wound sepsis[8, 9] and are occasionally complicated by fatal septicaemia[10], particularly after certain forms of cholangiography[11, 12]. It has also been suggested that there is an association between the presence of bacteria in the bile and postoperative sepsis[13].

John Hunter wrote *A Treatise on the Blood, Inflammation and Gun-shot Wounds*[14]. I propose to describe inflammations in wounds and the blood caused not by war but by micro-organisms in the bile.

Bacteria in the bile and postoperative sepsis

Bacteria in the bile at operation Biliary infection was found in 57 (31%) of 181 patients undergoing biliary surgery. The presence of bacteria in the bile was significantly ($P<0.01$) more common in patients requiring emergency operation (16/17; 94%) than in patients undergoing elective operations (41/164; 25%). Furthermore, the incidence of biliary infection among those requiring elective operation was higher in jaundiced patients (14/25; 56%) than in the non-jaundiced group (27/139; 19%), particularly when the jaundice was due to stones (10/14; 71%) rather than to malignant disease obstructing the bile ducts (4/11; 36%).

I have reviewed the morphology of 199 organisms in the bile from 136 consecutive patients who were not receiving antibiotic cover. The majority of the organisms (87%) were aerobic bacteria, particularly *Escherichia coli, Streptococcus faecalis,* and *Klebsiella aerogenes.* There were only 25 anaerobic organisms (13%) and *Bacteroides* spp. were extremely uncommon (see Table I). A single species of organism was isolated from the bile in only 38% of patients with biliary infection, 2, 3 or even 4 species occurring to-

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TABLE I *Bacteria in the bile: morphology of 199 organisms*

Aerobic: 174 (87%)	
Gram-positive: 42 (21%)	
Streptococcus faecalis	30
Beta-haemolytic streptococci	4
Streptococcus viridans	1
Staphylococcus aureus	3
Staphylococcus albus	4
Gram-negative: 132 (66%)	
Escherichia coli	77
Klebsiella aerogenes	22
Enterobacter spp.	8
Proteus spp.	13
Pseudomonas aeruginosa	4
Acinitobacter spp.	4
Serratia spp.	2
Aeromonas spp.	2
Anaerobic: 25 (13%)	
Gram-positive: 23 (12%)	
Clostridium welchii	16
Anaerobic streptococci	7
Gram-negative (1%)	
Bacteroides spp.	2

gether in most cases.

The incidence of bacteria throughout the biliary tract was determined in 71 consecutive patients. When the lumen of the gallbladder was infected the bile duct was always colonized by the same bacteria. In patients with bacteria in the gallbladder bile organisms were isolated from 80% of liver biopsy specimens, from 80% of duodenal aspirates, from the wall of the gallbladder in 88% of cases, and from the cystic lymph node in 60%. Conversely, when the gallbladder bile was sterile so were all other sites tested with the exception of the duodenum (52%) and the gallbladder wall (30% infected).

Bacteria in the T-tube bile Choledochotomy with T-tube drainage was performed on 50 patients. Although the bile was infected at operation in only 24 cases (48%), by the 5th postoperative day organisms were recovered from the T-tube drain in 40 (80%). The bacteria recovered from the T tube frequently differed from those found at operation. The bile remained sterile in only 10 cases (20%), 'new' organisms were recovered from the T tube in 25 (50%), and in only 15 cases (30%) did the T tube contain the same organisms as those previously isolated at operation. The 'new' bacteria found in the T tube were intestinal organisms and not surface pathogens, so it is unlikely that they can have been introduced from outside. I believe that many of the organisms colonizing a T tube are introduced into a germ-free biliary tract during the operation, either from the gallbladder wall during operative manipulation or during dilatation of the distal bile duct, when bacteria are introduced from the duodenum.

Over 10^5 organisms /ml were isolated from 90% of patients with biliary infection and in many cases counts exceeded 10^7 /ml both in operative bile samples and samples from the T tube (Fig. 1).

Wound sepsis and septicaemia after biliary operations Wound sepsis was defined as the presence of pus in the incision and was recorded in 20% of the 181 patients. There was a significantly higher incidence of wound sepsis after emergency surgery (41%) than after elective operation (18%). Further-

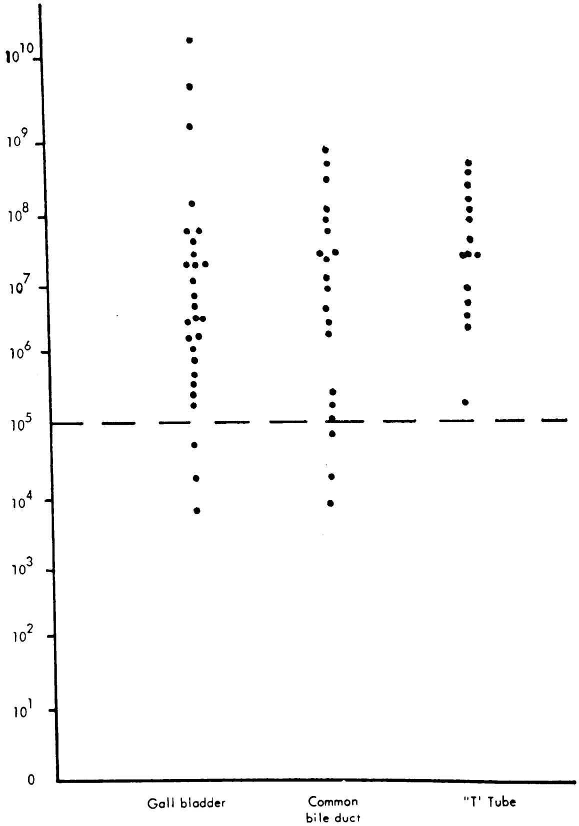

FIG. 1 *Viable counts of bacteria in bile.*

TABLE II *Incidence of wound sepsis and septicaemia*

	No of patients	*Wound sepsis*		*Septicaemia*	
A) *Emergency operation*	17	7	(41%)*	3	(18%)
B) *Elective operation*	164	29	(18%)	9	(6%)
1) Cholecystectomy alone	101	10	(10%)	1	(1%)
2) Choledochotomy	63	19	(31%)†	8	(12%)†
a) Stones	23	8	(35%)	5	(22%)
b) Carcinoma	13	4	(31%)	1	(8%)
c) No stones	27	7	(26%)	2	(7%)
Total	181	36	(20%)	12	(7%)

*$P<0.05$
†$P<0.01$

more, for elective operations the incidence of wound sepsis was 3 times greater after choledochotomy than after cholecystectomy alone (see Table II).

Septicaemia was defined as the presence of fever, rigors, or shock accompanied by a positive blood culture; this complication was recorded in 7% of patients. For elective operations the incidence of blood-stream sepsis was significantly higher after exploration of the bile duct (12%) than after cholecystectomy alone (1%).

Septicaemia was recorded in 27 (6.2%) of 436 patients who were not receiving antibiotic cover. Five patients developed septicaemia within 6 h of transhepatic cholangiography and 2 after endoscopic retrograde cholangiopancreatography. Septicaemia was also recorded in 2 patients after choledochoscopy and in 2 other patients in whom an operative cholangiogram had demonstrated cholangiovenous reflux due to impacted calculi in the bile duct. There were 7 episodes of rigors and cholangitis after T-tube cholangiography. Of the 27 patients with septicaemia, 9 developed endotoxic shock, complicated by acute renal failure in 6, and 5 died. This represents an overall mortality from septicaemia in patients undergoing biliary surgery of 1.5%.

Relationship between biliary infection and postoperative sepsis This relationship was investigated in 282 patients. When organisms were found in the bile at operation the incidence of wound sepsis (38%) and septicaemia (16%) was significantly greater than in patients with sterile bile (10% and 5% respectively.

After choledochotomy wound infection and septicaemia were significantly more common in patients with bacteria in the T tube (45% and 22% respectively) than in the small number in whom the bile remained sterile throughout the period of T-tube drainage (7% and nil respectively).

A comparison was also made between the types of organism in the bile and those infecting the wound or blood stream afterwards. After biliary operations 64% of the wound infections and 90% of the episodes of septicaemia were caused by organisms which had been previously isolated from the bile.

Influence of prophylactic antibiotic cover

Antibiotic sensitivities The sensitivities of organisms isolated from the bile to 5 antibiotics are listed in Table III. The Gram-negative aerobic organisms were uniformly sensitive to gentamicin. Although many of the Gram-positive aerobic bacteria and the anaerobic bacteria were resistant to gentamicin, all except 2 were sensitive to ampicillin.

Relative merits of bile and serum levels of an antibiotic It has been suggested that antibiotics with which high bile levels can be achieved should be used both to eliminate bacteria from the biliary tract and also to minimize the risk of postoperative sepsis[15, 16]. The majority of patients with infected bile, however, have obstructive biliary disease, and there are numerous reports to indicate that adequate levels of antibiotic are not reached in the bile in patients with obstruction of the bile duct or cystic duct[17-19].

TABLE III *Sensitivity to 5 antibiotics of 170 organisms isolated from bile (310 patients).*

Antibiotic	*All organisms*	*Aerobic* Gram +ve (24)	Gram −ve (131)	*Anaerobic* (15)
Gentamicin	85%	56%	100%	0%
Cephaloridine	76%	58%	71%	66%
Tetracycline	54%	58%	51%	74%
Rifampicin	50%	67%	46%	66%
Ampicillin	43%	93%	27%	100%

To determine the influence of prophylactic antibiotic therapy on postoperative sepsis a controlled trial was carried out on 150 consecutive patients undergoing biliary operations[20]. Each patient was allocated at random to one of three groups: (1) 50 patients received gentamicin, an antibiotic with high serum but low bile levels; (2) 50 patients received no antibiotic and acted as controls; and (3) 50 patients received rifamide, an antibiotic excreted almost entirely into the bile. Patients in both treated groups received their first dose of antibiotic at 6 a.m. on the morning of operation and a second dose in the anaesthetic room. After operation the antibiotics were continued 8-hourly for 5 days. The doses of gentamicin and rifamide were 80 mg and 150 mg respectively, given intramuscularly throughout. The randomization was stratified to provide equal numbers of jaundiced patients in the three groups (11 each).

The minimum inhibitory concentration (MIC) of gentamicin was measured in 83 patients with biliary infection and 2.0 mg/l was found to be a sufficient concentration to inhibit over 80% of organisms in the bile. Therefore twice this figure (4 mg/l) was arbitrarily considered to provide an 'adequate' level of gentamicin in patients with biliary disease. The concentrations of gentamicin present in the gallbladder, common bile duct, and serum at operation are shown in Figure 2. Whereas 'adequate' levels of gentamicin were found in the bile in only 2 cases, 'adequate' serum concentrations were present in almost 90%.

The MIC of rifamide was determined in 38 patients with biliary infection. Over 80% of the organisms in the bile were inhibited by 31.25 mg/l so twice this level (62.5 mg/l) was regarded as 'adequate' concentration of rifamide for patients with biliary disease. The bile and serum concentrations of rifamide found at operation are shown in Figure 3. Serum levels of rifamide were invariably too low to be of therapeutic value and although extremely high bile levels were achieved in most patients in whom there was no evidence of obstruction to the biliary tract, 'inadequate' bile levels were present in all except 2 patients with obstructive biliary disease (that is, cystic

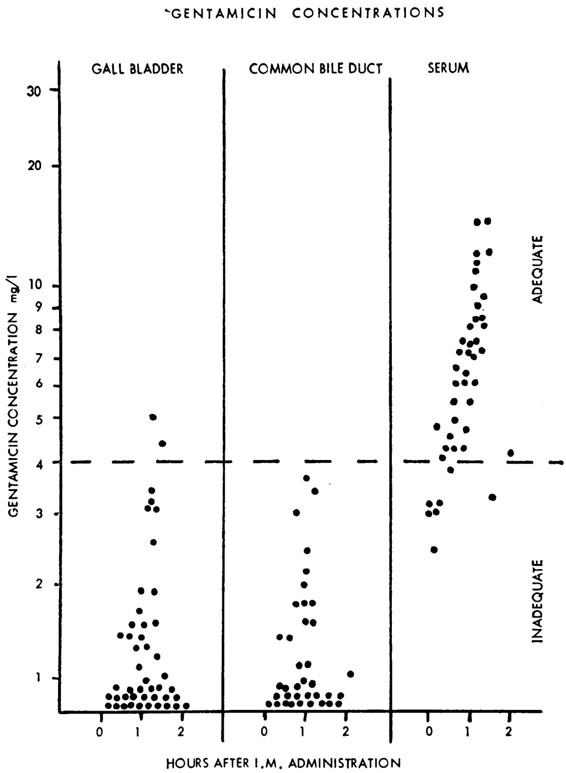

FIG. 2 *Concentrations of gentamicin in bile and serum at operation.*

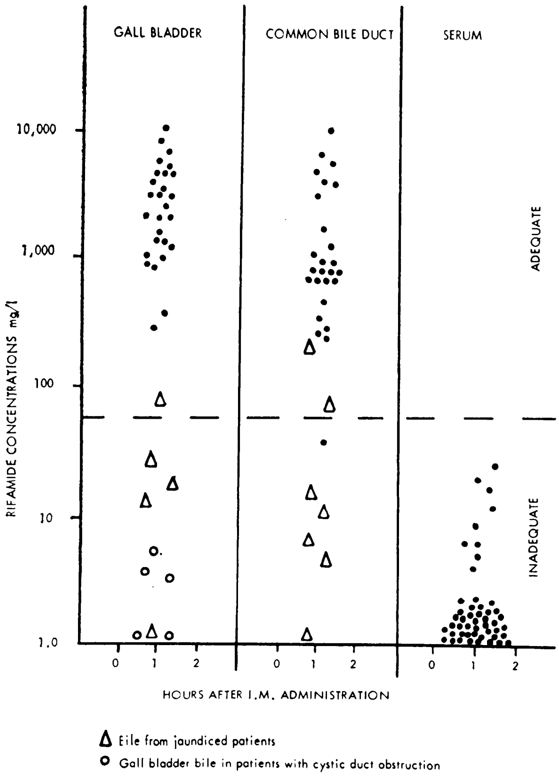

FIG. 3 *Concentrations of rifamide in bile and serum at operation.*

duct or bile duct occlusion). These results indicate that although high concentrations of rifamide are normally achieved in the bile, in patients with biliary obstruction, in whom biliary infection and postoperative sepsis are most likely to occur, both serum and bile levels of the antibiotic are too low to be of therapeutic value.

In view of these findings it is hardly surprising that biliary infection was not significantly reduced by either antibiotic (Table IV). Of the two regimens of prophylactic antibiotic therapy, a significant reduction in wound sepsis and septicaemia was demonstrated only in the patients receiving the antibiotic with satisfactory serum levels (gentamicin). Furthermore, the only patients who benefited from antibiotic cover were those in whom the bile contained organisms at the time of operation. I would suggest that an effective bactericidal antibiotic with which satisfactory serum levels can be achieved is more likely to be effective, particularly in patients with obstructive biliary disease, than an antibiotic which is excreted almost entirely into the bile.

Detection of patients with biliary infection requiring antibiotic cover If antibiotics are given to all patients undergoing an operation on the biliary tract there will be a large proportion of patients in whom the bile is sterile and who will therefore receive unnecessary antibiotic cover. I have already indicated that if no antibiotics are given there is an unacceptably high incidence of postoperative sepsis. Patients requiring antibiotic cover are those in whom the bile is infected at operation. I have attempted to identify patients with biliary infection either before or during operation. Unfortunately, such indices as the preoperative white cell count, the nitroblue tetrazolium test, and preoperative blood cultures are all unsatisfactory.

In Birmingham we have found that there are three methods of identifying patients with bacteria in the bile: these are: (1) preoperative duodenal aspiration, (2) an analysis of the clinical presentation and operative findings of patients, and (3) the use of operative Gram staining of the bile.

1) Collections of duodenal aspirate were obtained for culture and viable counts from 47 patients before operation; the results were later compared with the operative bile cultures. Small numbers of oral commensals were frequently isolated from the duodenum even though the bile was sterile. Duodenal aspirates were therefore considered likely to have been contaminated during the passage of the Ryle's tube unless the duodenal collections contained more than 10^5 organisms/ml. When this criterion was applied preoperative duodenal aspirates accurately defined the presence and type

TABLE IV *Results of prophylactic antibiotic therapy in biliary surgery*

	Gentamicin (50)	*Controls* (50)	*Rifamide* (50)
Biliary infection	12 (24%)	19 (38%)	10 (20%)
Wound sepsis	3 (6%)*	11 (22%)	5 (10%)
Bacteraemia	1 (2%)*	7 (14%)	4 (8%)

*$P<0.05$ compared with controls

of biliary organisms in 75% of patients, there being a 6% incidence of false positive findings and an 11% incidence of false negative results, while the organism was incorrectly identified in 8% of patients. Preoperative duodenal aspiration is, however, time-consuming, and satisfactory collections of duodenal fluid may be difficult to obtain.

2) A multivariate analysis was used in an attempt to define patients with biliary infection by means of their clinical presentation and operative findings[21]. A cluster analysis of 17 clinical and 10 operative variables was used for this study. Five divisions formed 6 groups which were classified as high- or low-risk depending on the likelihood of organisms being cultured from the bile. There was one group of low-risk patients (136 cases) and 5 high-risk groups (totalling 45 patients). The incidence of biliary infection was 67% in the high-risk and 19% in the low-risk population, and this represents a highly significant difference. To determine the factors responsible for separating the high- and low-risk groups the incidences of the 27 variables were compared in both populations. Using a probability value of less than 0.1%, 8 variables were found to be much more common in the high-risk than in the low-risk group. These were: (*a*) age over 70 years, (*b*) jaundice present at operation, (*c*) recent history of rigors, (*d*) emergency operation (*e*) operation performed within 4 weeks of an acute admission for biliary colic, jaundice, or cholecystitis, (*f*) history of previous biliary operation, (*g*) presence of choledocholithiasis, and (*h*) presence of bile duct obstruction. However, if these criteria alone were used for identifying patients with bacteria in the bile 33% would receive unnecessary antibiotic cover.

3) The accuracy of operative Gram staining of bile was examined in 198 patients. Once the diagnosis of biliary disease had been established at operation a sample of bile was aspirated from the gallbladder and sent to the laboratory for immediate Gram staining and then plated out for culture. The results of examination of the Gram-stained preparation were telephoned to the operating theatre within 20 min of collection. The overall accuracy of this technique in detecting the presence and type of biliary organisms was 77%, with a 12% incidence of false positive findings (largely due to epithelial debris) and a 7% incidence of false negative results, while the organism was incorrectly identified by the Gram stain in 4%. Although Gram staining of bile is fairly accurate as a means of detecting patients with biliary infection, the results are not known until the operation is being performed and it has been argued that antibiotics should be given before rather than during operation[22].

Results of selective antibiotic cover

I have tried to select patients requiring antibiotic cover during the operation on the basis of the results of immediate Gram staining of bile. If Gram-positive bacteria are seen ampicillin is given, if Gram-negative organisms are reported gentamicin is used, if both Gram-positive and Gram-negative bacteria are present both antibiotics are given, and if no organisms are seen patients receive no antibiotic. The antibiotic is administered by bolus intravenous injection by the anaesthetist as soon as the Gram-stain results are known, usually during operative cholangiography and invariably before wound closure. The antibiotics are then continued for 5 days. Using this policy we found that prophylactic antibiotics had been avoided in 59% of 119 patients. The choice of peroperative antibiotic cover was later compared with the final bile cultures and we found that 82% of patients had received appropriate cover. Only 12% received unnecessary antibiotics, but there were 7% with biliary infection who either received the wrong antibiotic or none at all.

I have also compared the clinical results in 119 patients who were given 'selective antibiotic cover' on the basis of the results of immediate Gram staining of bile with those in a group of 101 matched controls who received no antibiotic cover. There was a significant reduction of wound sepsis in the 'selectively treated' group (7%) compared with the controls (22%) and the incidence of septicaemia was also reduced (from 8% to 2%). I conclude that peroperative antibiotic cover will reduce the morbidity of biliary operations. Furthermore, unnecessary antibiotic cover can be avoided by providing cover only to patients in whom immediate Gram staining of bile has demonstrated that the biliary tract is infected.

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References

1 Hunter, J (1861) *Essays and Observations,* ed. Owen, R.
2 Welch, W H (1891) *Johns Hopkins Medical Journal, 2,* 121.
3 Edlund, Y, Mollstedt, B O, and Ouchterlony, O (1958) *Acta chirurgica Scandinavica, 116,* 461.
4 Engstrom, J, Groth, C G, Lundh, L G, and Lonnquist, B (1792) *Acta chirurgica Scandinavica, 138,* 357.
5 Flemma, R J, Flint, L M, Osterhout, S, and Shingleton, W W (1967) *Annals of Surgery, 166,* 563.
6 Scott, A J (1971) *Gut, 12,* 487.
7 Watson, J F (1969) *Military Medicine, 134,* 416.
8 Mason, G R (1968) *Archives of Surgery, 97,* 533.
9 *Lancet* (1960) *2,* 659.
10 Jones, E A, Sherlock, S, and Crowley, N (1967) *Postgraduate Medical Journal, 43,* 7.
11 Blumgart, L H, Cotton, P B, Burwood, R, Lawrie, B, Salmon, P, Davies, G T, Beales, J S M, Skirving, A, and Read, A E (1972) *Lancet, 2,* 1269.
12 Keighley, M R B, Kelly, J P, and Wilson, G (1973) *British Medical Journal, 3,* 147.
13 Robson, M C, Bogart, J C, and Heggers, J P (1970) *Surgery, 68,* 471.
14 Hunter, J (1794) *A Treatise on the Blood, Inflammation and Gun-shot Wounds,* p. 152. London, Nichol.
15 Acocella, G, Matiussi, R, Nicolis, F B, Pallanza, R, and Tenconi, L T (1968) *Gut, 9,* 536.
16 Stratford, B C (1966) *Medical Journal of Australia, 1,* 7.
17 Khan, G A, and Scott, A J (1967) *British Journal of Pharmacology, 31,* 566.
18 Sales, J E L, Sutcliffe, M, and O'Grady, F (1972) *British Medical Journal, 3,* 441.
19 Mortimore, P R, Mackie, D B, and Haynes, S (1969) *British Medical Journal, 3,* 88.
20 Keighley, M R B, Drysdale, R B, Quoraishi, A H, Burdon, D W, and Alexander-Williams, J (1976) *Gut, 17,* 495.
21 Keighley, M R B, Flinn, R, and Alexander-Williams, J (1976) *British Journal of Surgery, 63,* 528.
22 Burke, J F (1961) *Surgery, 50,* 161.