A microbiological and morphological study of blocked biliary stents

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Biliary stent blockage represents the main limitation of the use of such devices in relieving obstructive jaundice due to a variety of malignant and benign conditions. Microbiological and morphological analysis of the occluding sludge present on the inner surface of 30 biliary stents was performed to evaluate the different components of such material and the effect of the antibiotic treatment on the biofilm formation. A highly organized biofilm, constituted by microbial cells embedded in an amorphous matrix together with crystallized bile salts, was observed *Enterococcus spp*. represented the most common isolate from both occluded and non-occluded stents. The antibiotic therapy, while selecting for multi-resistant bacteria and fungi, might possibly delay the biofilm formation. *Key words*: biliary stents, microbial colonization, biofilm.

INTRODUCTION

Biliary stent placement is now a well established procedure for treating a variety of malignant and benign conditions leading to the obstruction of biliary and pancreatic ducts. However, late recurrent jaundice and cholangitis due to stent blockage are major limitations to the use of this procedure, stent occlusion occurring in 20–30% of cases within 3 months of primary placement (1, 2). A role for bacteria in the early occlusion of biliary stents has been suggested (3, 4), possibly correlated to the unevenness of the polymeric surface (5). As a matter of fact, colonization of indwelling biliary stents is almost inevitable as microorganisms can reach the biliary system either through the sphincters of Oddi or via the hematogenous route from the portal venous system (6, 7). In this view, it may be of importance to select appropriate antibiotic prophylaxis and/or therapy to prevent or to delay the formation of the biliary sludge responsible for the stent occlusion.

In this study, the authors have examined a number of biliary stents, explanted either because of occlusion or for other reasons, to evaluate the morphological and microbiological aspects of the occluding material and the effect of antibiotic treatment on the biofilm formation.

MATERIAL AND METHODS

Patients and biliary stents

In an 18-months period, 30 biliary stents explanted from 26 patients suffering from biliary obstruction, were examined. Patients were 14 males and 12 females, age ranging from 46 to 89 years (mean 66.7 years, median 70 years).

Thirteen of these patients presented with a malignant obstructive jaundice: five pancreatic; five common biliary duct and two gallbladder adenocarcinomas, and one metastatic lesion from a hepatic adenocarcinoma. The other 13 patients suffered from benign biliary stenosis: seven iatrogenic strictures; three common bile duct stone; two chronic pancreatitis and one sclerosing colangitis.

All stents were 10-French gauge either of polyurethane, PVC or Teflon. Eighteen stents were explanted because of blockage, while 12 were removed because of dislocation, recovery or end of treatment.

Stent processing

Right after explantation, 5 mm of both the duodenal and biliary ends of the stents were cut under sterile conditions, further divided into two sections, and placed either into phosphate buffered saline (PBS) or in 2.5% cacodylate-buffered glutaraldehyde. The remaining part was placed in PBS and stored at −70°C for eventual additional processing.

Microbiological analysis. Stents in PBS were sonicated for 10 min at 2 μA in ice (Soniprep 150, MSE) to dislocate the adherent microorganisms; 0.1 and 0.01 ml of the

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effluent were plated on non-selective media and incubated under aerobic and anaerobic conditions for 24–48 h. Isolated microorganisms were counted, identified at species level according to standard biochemical tests (8), and stored at −20°C.

Electron microscopy. Segments of the biliary and of the duodenal ends of the stents fixed in cacodylate-buffered 2.5% glutaraldehyde, were bisected along their long axis and postfixed in 1% osmium tetroxide. Samples were dehydrated either in ethanol solution before critical point drying or air dryed at 40°C for 2 h. After gold sputter coating, samples were examined by a 902 Cambridge electron microscope.

Adherence to polystyrene. *Enteroccoccus spp*. and *Staphylococcus spp*. strains isolated in the course of the study were characterized for their adherence ability to plastics. Briefly, overnight cultures were diluted 1:100 in TSB in 96-well polystyrene plates. After incubation at 37°C, plates were washed three times with PBS and the adherent bacteria stained with Hucker crystal violet. Optical density was measured by a Novapath™ Microplate Reader (BioRad) at 570 nm wavelength; optical densities above 0.240 (evaluated as being the O.D. of control wells ± 3 SD) identified adherent bacteria.

Statistical evaluation. Differences were analyzed for significance by the χ^2 test.

RESULTS

Implantation time of the stents was 58 days (median) with a range of 2–380 days. In particular, stents removed because of occlusion had been in place for a median time of 150 days vs 16 days of the stents explanted for reason other than blockage.

Microbiological examination of the 18 blocked stents revealed microbial growth in 15 cases (83.3%), while microorganisms were recovered from six of the 12 non-occluded stents (χ^2 3.6, $p = 0.05$). No relevant differences of microbial growth between the duodenal and the biliary ends of the stents were detected.

The median implantation time was 58 days for the colonized stents vs 8 days for the sterile ones. In 21 cases, antibiotic treatment was given at the time of explantation: ampicillin 1; cephalosporin 7 (ceftazidime 3, ceftriaxone 3 and cefotetan 1); gentamicin 2; pefloxacin 2; vancomycin 1 and piperacillin plus aztreonam 2. Antibiotic therapy was undefined in six cases. In four cases the antibiotic treatment lasted for the whole implantation period of the stent; of these three were sterile.

Out of the 21 patients under antibiotic treatment, obstruction was present in 15 (71.4%) cases, while colonization was detected in 13 (62%) cases. A statistical evaluation of these data was not performed because of the small number involved.

Table I

Microbial flora recovered from biliary stents

Isolates	No. of strains		
	Occluded stents	Non-occluded stents	Total
Gram-positive			
E. faecalis	9	4	13
E. faecium	5	1	6
E. raffinosus	2	–	2
E. gallinarum	2	–	2
E. durans	–	1	1
Str. bovis	1	–	1
Staph. aureus	1	–	1
Staph. epidermidis	4	–	4
Staph. capitis	1	–	1
Bacillus spp.	–	1	1
Pediococcus spp.	1	–	1
Gram-negative			
E. coli	4	–	4
K. oxytoca	2	–	2
K. pneumoniae	1	–	1
E. cloacae	1	1	2
C. freundi	1	–	1
Ps. putida	1	–	1
Anaerobes			
Veillonella spp.	1	–	1
Mycetes			
Candida spp.	4	1	5
Total	41	9	50

From 21 colonized stents, 50 isolates belonging to 19 different species were recovered and identified as reported in Table I: 11 were gram-positive, six were gram-negative. Also, one anaerobic and one fungal strain were isolated. Forty-one isolates were from the 18 occluded stents (2.27 strains/stent) and nine were from the 12 non-occluded stents (0.75 strains/stent). In Table II the quantitative microbial analysis is reported.

Sixteen out of 24 *Enterococcus spp*. strains, and the six *Staphylococcus spp*. strains isolated from the explanted stents showed preferential adherence to polystyrene when examined for their adherence to plastic.

Direct examination of the stents showed a yellow-brown material adhering to the inner surface of the explanted stents, sometimes protruding from the terminal end.

Table II

Quantitative analysis of bacterial growth

Bacterial count	Occluded stents	Non-occluded stents
>10.000 cfu/ml	7 (38.8%)	1 (8.3%)
1–10.000 cfu/ml	8 (44.4%)	5 (41.6%)
Sterile stents	3 (16.6%)	6 (50%)

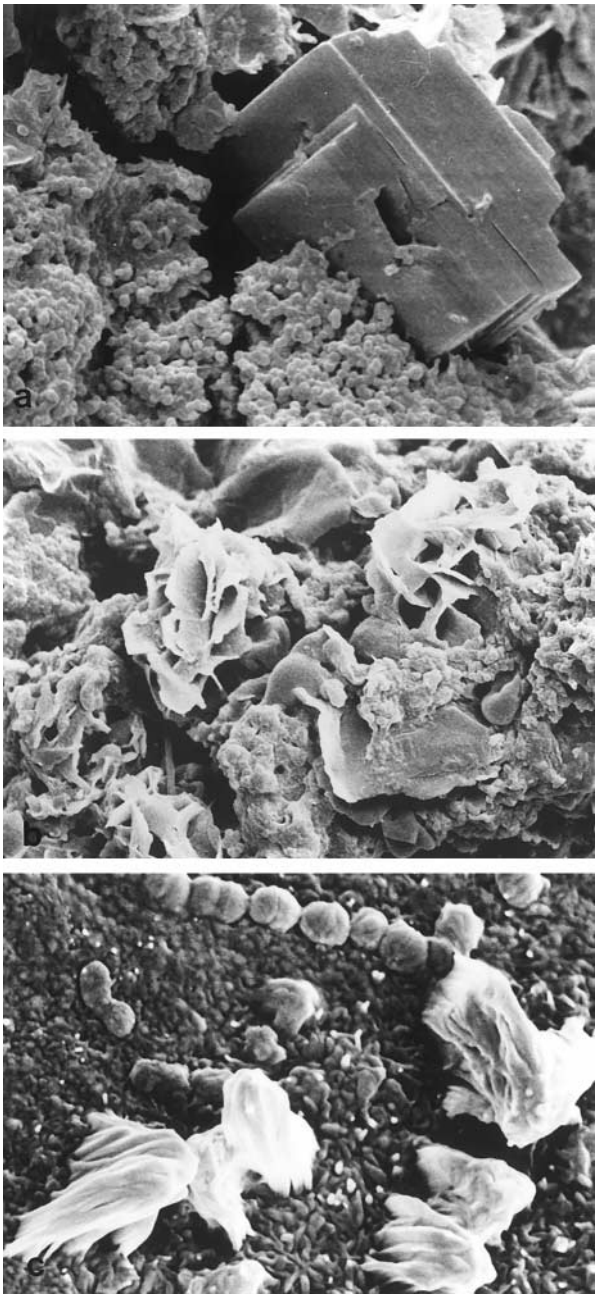

Fig. 1. Scanning electron micrographs of the material deposited on the inner surface of a blocked stent show crystals of various morphology visible embedded within an amorphous matrix. Based on the morphotypes described in the literature, the parallel-edged crystals were tentatively identified as calcium bilirubinate (a), the rosettes-arranged scales as calcium palmitate (b) and the flock-shaped ones as (c) cholesterol crystals. In (a) and (c) coccoid cells are also visible. a,c) 2500 × ; 8400 × .

At scanning electron microscopy examination, both the inner and the outer surfaces of the stents were covered to various degrees by a biofilm consisting of microbial cells and crystals of different morphology, embedded in a thick layer of amorphous material (Fig. 1). According to the morphotypes described in the literature (4, 9, 10), crystals were tentatively identified as calcium bilirubinate crystals (Fig. 1a), calcium palmitate rosettes (Fig. 1b) or cholesterol crystals (Fig. 1c). Air drying allowed observation of both the organic and the non-organic features of the biofilm in the same sample, although the preservation of the microbial cells was not optimal (Fig. 2). In comparison to unused stents examined as control, gross morphological alterations of the stent surface were not observed even after the longest time of implantation.

In six cases, coccoid or bacillary forms deeply, embedded into the organic matrix, were observed by SEM,

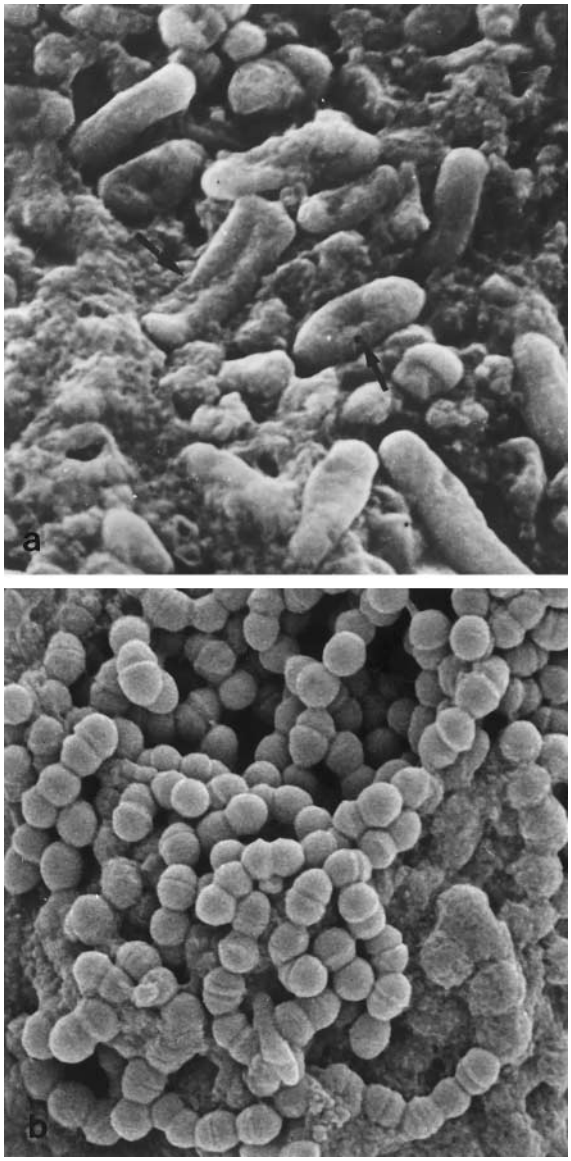

Fig. 2. The occluding material present along the inner stent surface presented with a mixed flora of cocci and bacilli. Air drying of the samples, instead of the routine critical point drying processing, allowed a still satisfactory preservation of the microbial cells, although bacilli (a) showed morphological alterations (arrows) more frequently than coccoid cells (b). (a) 8000 × ; (b) 14000 × .

corresponding to a negative response from the microbiologists. Samples were then retrieved from storage and submitted to a further sonication cycle; in all these cases but one, this procedure resulted in the recovery and identification of the bacterial species involved.

DISCUSSION

Blockage is a common complication of the use of endoscopic biliary stents utilized to relieve obstructive diseases of the biliary tract. Stent occlusion is basically caused by biliary sludge formed by a mixture of bacteria, protein and salts constituents of the bile (4). Even a short-term delay in the process of blockage may prolong stent patency and reduce the morbidity from recurrent jaundice and cholangitis.

The authors examined 30 stents, explanted for either blockage or other reasons, to evaluate the composition of the microbial flora forming the occluding sludge. Bacteria of the genus *Enterococcus*, *E. faecalis* in particular, were the most frequently isolated (24 out of 50 microbial isolates) and were recovered from both the occluded and non-occluded stents. *Escherichia coli* was the most common gram-negative isolate, and was recovered from occluded stents only. *Candida spp*. was the only non-bacterial isolate. The isolation of *Enterococcus spp*. as the most frequent strain only partially agrees with previous reports describing *E. coli* as the most common genus from blocked biliary stents (4) and *E. coli* and *E. faecalis* only from suppurative cholangitis (11–13). Such bacterial composition reflects the duodenal origin of the colonizing flora, which reaches the biliary system primarily through the sphincter of Oddi (7).

The present data indicate that bacteria represent the main constituent of the occluding sludge since the blocked stents presented bacterial colonization in a higher percentage (83 vs 50%), a higher number of strains (2.27 vs 0.75) and the bacterial counts were higher (>10.000 cfu/ml) more frequently in this group compared to that of the non-occluded stents. Such conclusion was also drawn by Speer et al. (4), based on the observation of the highly organized structure of the bacterial biofilm.

The antibiotic treatment seemed to quantitatively reduce the microbial colonization; in this view, for short-term stents, an antibiotic coverage lasting the whole implantation period may effectively delay the sludge formation, thus prolonging the stent life. As a matter of fact, of the four patients who had received antibiotic treatment for the whole implantation period, three remained sterile and pervious.

The selection of the antibiotics to be used in patients carrying endoscopic stents should take into consideration their role in delaying microbial colonization and thus prolonging stent effectiveness.

On the other hand, antibiotic treatment also selects the colonizing flora; in fact, the antibiotics most frequently included in the therapeutic protocol (cephalosporin, aminoglycoside, and quinolones) reflect and explain the prevalence in the duodenal flora of the multi-resistant bacteria of the genus *Enterococcus*, which were all vancomycin-sensitive but resistant to third-generation cephalosporins and quinolones, and the *Candida spp*. isolation observed in the course of the study.

Rather than considering a long-lasting antibiotic coverage, safer and less cumbersome treatments such as silver-coating of the stents (14), elimination of the side holes (15) and the use of preparations of bile salts (16) have been indicated to prevent microbial adhesion.

Morphological examination of the occluding sludge revealed a highly organized biofilm formed by crystals of various forms and microbial cells embedded within a matrix of probable bacterial origin. The use of the air drying procedure was preferred for routine examination since the exposure to organic solvents extracted parts of the crystals from the biofilm. Even with this preparation, the relative contributions of organic biofilm and crystalline deposits to the clogging material are difficult to judge; however, SEM observations seem to confirm a prominent role of colonizing bacteria. Additional support to this view is provided by the result that the majority of *Enterococcus* strains and all the six staphylococcal strains isolated in this study showed a preferential adherence to plastic, a character which well correlates with biofilm formation on medical devices (17).

As even a short-term delay in the process of blockage may prolong stent patency and thus be helpful especially in patients with only a limited survival time, a careful selection of the antibiotics used during stent implant should be performed while searching for safer, less expensive, procedures.

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