Annals of Gastroenterology (2014) **27,** 399-403

Isolated microorganisms in plastic biliary stents placed for benign and malignant diseases

Paris Basioukas[a], Antonios Vezakis[b], Olympia Zarkotou[c], Georgios Fragulidis[b], Katerina Themeli-Digalaki[c], Spyros Rizos[a], Andreas Polydorou[b]

Tzaneio General Hospital, Piraeus; University of Athens, Aretaieio Hospital, Greece

Abstract

Background Biliary stenting is a well-established method to treat patients with malignant and benign biliary diseases. However, occlusion of plastic biliary stents is considered as a drawback and bacterial colonization seems to be the key factor in this process.

Methods During a 3-year period, 51 plastic biliary stents were extracted from 42 patients. Twenty three stents were inserted for treating malignant and 28 for benign diseases. Stent samples were taken under a strict protocol, and were immediately sent to microbiological laboratory for culturing.

Results A polymicrobial growth was present in nearly all stents. The most frequently isolated organisms were *Enterococcus spp* (74%), *Escherichia coli* (*E. coli*) (62%), and *Klebsiella spp* (58%). *E. coli* was more frequently encountered in benign vs. malignant disease (78% vs. 43%, P<0.05). *Klebsiella spp, Pseudomonas spp*, and *Candida spp* were more frequently isolated in occluded vs. non-occluded stents, 68% vs. 37%, 22% vs. 0 and 40% vs. 6% respectively (P<0.05). *E. coli* and *Pseudomonas spp* had 34% and 50% resistance rate to quinolones respectively. *Enterobacter spp* expressed Amp-C derepression in 35%. *Enterococcus spp*, *Klebsiella spp* and *Pseudomonas spp* had a low resistance rate.

Conclusion *Enterococcus spp*, *E. coli* and *Klebsiella spp* are the most frequently associated organisms in plastic biliary stents. In occluded stents *Pseudomonas spp* and *Candida spp* should be taken into account. Quinolones may not be adequate for the treatment of cholangitis associated with stent occlusion. In patients under chemotherapy for malignancy and stent occlusion-related biliary sepsis, antifungal and enterococcal covering should be considered.

Keywords Biliary stents, occlusion, microorganisms, microbial growth, antibiotic resistance

Ann Gastroenterol 2014; 27 (4): 399-403

Introduction

Since its first application in 1968. endoscopic retrograde cholangiopancreatography (ERCP) [1] remains an effective procedure for diagnosis and mainly for treating diseases of biliary and pancreatic tracts. The evolution of other non-invasive imaging techniques, such as magnetic resonance cholangiopancreatography (MRCP) [2] and endoscopic ultrasound (EUS) [3], has limited the diagnostic prospective of ERCP [4]. However, endoscopic sphincterotomy, stone extraction and stent placement have established ERCP as a gold standard treatment for a variety of malignant and benign diseases of biliary and pancreatic ducts [5]. When plastic stents are chosen to maintain bile duct patency, stent occlusion with consequent bile stasis and cholangitis constitutes one of the major late complications [6,7]. The elimination of the anti-microbial barrier of Oddi [8] and the low pressure in common bile duct, due to endoscopic sphincterotomy and endoprosthesis insertion leads to duodenal reflux, allowing bacterial colonization and biofilm formation, resulting to stent occlusion [7,9,10]. This study was performed to identify the spectrum of microbial flora involved in endoprosthesis colonization and biofilm formation, as well as to evaluate their resistance to advanced antibiotic therapy.

[a]First Department of Surgery, Tzaneio General Hospital, Piraeus (Paris Basioukas, Spyros Rizos); [b]Second Department of Surgery and Endoscopic Unit, University of Athens, Aretaieio Hospital, Athens (Antonios Vezakis, George Fragulidis, Andreas Polydorou); [c]Department of Microbiology, Tzaneio General Hospital, Piraeus (Olympia Zarkotou, Katerina Themeli-Digalaki), Greece

Conflict of Interest: None

Correspondence to: Paris Basioukas, MD, First Department of Surgery, Tzaneio General Hospital, Afentouli 1, 18536 Piraeus, Greece, Tel.: +30 6974 352424, e-mail: pbasioukas@gmail.com

Received 26 May 2014; accepted 11 June 2014

Patients and methods

During a 3-year period, 51 plastic biliary stents were extracted from 42 patients. There were 25 female and 17 male patients with a median age of 71 years (range 39-92). Twenty three stents were inserted for treating malignant disease and 28 for benign disease (Table 1). A total of 31 straight polyurethane stents with side flaps (20 had a diameter of 10-Fr and 11 of 7-Fr) and 20 polyurethane pig-tail stents with side holes (7-Fr) (Wilson-Cook Medical Inc.) were extracted. Stent extraction was decided on a scheduled basis up to 3 months after placement for benign disease or if clinical signs of stent clogging such as cholangitis, recurrent jaundice or biliary colic with elevated liver function tests developed.

All patients with symptoms of stent occlusion had been administered antibiotics for various lengths of time before ERCP. Patients scheduled for stent removal were given a single dose of antibiotic before ERCP, usually cefuroxime or ciprofloxacin.

Stent preparation

Under sterile conditions (surgical gloves) the extracted stents were cannulated using a sterile 21 G vein catheter, 10 cc of normal saline was injected and the collected lavage was distilled in a sterile culture tube and was immediately sent to the microbiological laboratory for culturing.

Microbiological study

The sample was cultured in blood agar, MacConkey agar (for selective isolation of Gram negative bacterial species) and Columbia agar (for selective isolation of anaerobic bacterial species). The identification of the isolated bacterial species, as well as the minimum inhibitory concentration (MIC) specification was based on Vitek 2 Comact system (bioMerieux, France) and auxiliary on API (bioMerieux, France) and BBL Crystal (BD) systems. Additionally, the confirmation of specific resistance phenotypes (e.g., extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs), AmpC type β-lactamases) was measured by Etest (AB Biodisk, Sweden).

Table 1 Main indication for the endoscopic intervention

Diagnosis	N of stents
Malignant disease	
Pancreatic cancer	15
Cholangiocarcinoma	4
Gallbladder cancer	2
Ampullary cancer	1
Metastatic lesion from colon cancer	1
Benign disease	
Choledocholithiasis	21
Iatrogenic injuries	5
Primary sclerosing cholangitis	2

Statistical analysis

Data from categorical variables were compared using the chi-square test or the Fisher's exact test. Continuous variables were compared using the Mann-Whitney test. All P-values were based on two-tailed tests. A P-value <0.05 was considered significant. Analysis was performed with Minitab 16 statistical software.

Results

Sixteen stents were schedule explanted and 35 because of clinical signs of occlusion. Fifty stents were endoscopically retrieved and one surgically during a Whipple's procedure. The median stent time to removal was 90 days (range, 9-730).

All our samples were positive for bacterial growth. Monomicrobial growth was present in two stents, whilst polymicrobial in all the remaining.

There were 162 growths from 16 different species. The median number of bacteria per stent was 3 (range, 1-6). There was statistical corellation between stent remaining time and number of organisms isolated (P<0.05) (Fig. 1). Table 2 shows the distribution of different microbial species in the stents. The most frequently encountered organisms were *Enterococcus spp* (74%), *Escherichia coli* (*E. coli*) (62%) and *Klebsiella spp* (58%).

There were no statistical differences between the organisms isolated between straight vs. pig-tail stents (P>0.05) and 10-Fr vs. 7-Fr stents (P>0.05). *E. coli* was more frequently encountered in benign disease vs. malignant disease (78% vs.43%; P<0.05). There was no statistical difference in the other organisms between malignant and benign disease (P>0.05). *Klebsiella spp*, *Pseudomonas spp*, and *Candida spp*

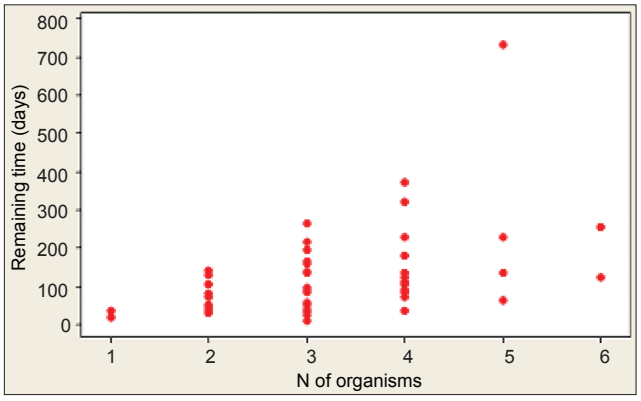

Figure 1 Scatterplot of stent duration in place vs. number of organisms
Pearson correlation of remaining time (days) and N of organisms=0,449
P-Value=0.001

were more frequently isolated in occluded vs. non-occluded stents, 68% vs. 37%, 22% vs. 0 and 40% vs. 6% respectively (P<0.05) (Table 3).

There was no statistical difference between organisms, as regards stent duration in place (P>0.05) (Table 4).

The screening for potential resistance of bacteria was performed by defining specific type β-lactamases (e.g. ESBLs, AmpC), carvapenemases (e.g. MBLs, *Klebsiella pneumoniae* carbapenemase-KPC) and resistance to advanced antibiotics (quinolones and vancomycin) (Table 5). Eleven of 32 (34%) isolated *E. coli* strains, and 4 of 8 (50%) *Pseudomonas spp* strains were resistant to quinolones. Five of 14 (35%) isolated *Enterobacter spp* strains expressed Amp-C derepression and were highly resistant. *Enterococcus spp* and *Klebsiella spp* had a low resistance rate.

There was no statistical difference in the resistance rate for *E. coli* and *Enterobacter* species, between occluded and non-occluded stents (P>0.05), and between malignant and benign diseases (P>0.05).

Discussion

The occlusion of endoscopically placed plastic biliary stents is the most frequent cause of biliary infection and recurrent jaundice, increasing morbidity and health care cost due to the need of stent exchange [9]. The occlusion mechanism is primarily based on bacterial colonization, as intestinal bacterial flora has ascending access to the biliary system due to elimination of the barrier function of the sphincter of Oddi. Subsequent biofilm formation on the inner surface of the stent by amorphous sludge from microbial byproducts, proteins, dietary fibers and biliary salts induce stent lumen encasement [11-14]. The outer surface of the distal end of the stent comes in direct contact with the duodenal lumen and our preparation technique was suggested to obtain samples from the inner surface where the bacteria are attached and the biofilm is formatted. However, a limitation is that contamination via the endoscope, when it is passed through the duodenum, or via cross-transmission between different patients cannot be excluded, despite vigilant disinfection.

A polymicrobial growth in bile or stents is quite common with an incidence ranging from 8 to 67% [15-17]. Nearly all our stents (96%) had polymicrobial growth. This high percentage may be due to the way our samples were obtained. The number of bacteria isolated per stent was in correlation with stent duration in place (P<0.05), and the longer duration of stent (P<0.05). On the contrary, the type of bacteria isolated was not related to stent duration (P>0.05).

Microbial organisms isolated from biliary stents include both aerobic and anaerobic species, as well as fungi [9]. *Enterococcus spp*, *E. coli*, and *Klebsiella spp* are the most common bacteria isolated from the sludge removed from biliary stents. However, the ratio between the isolated organisms varies in different studies, probably depending on either the portion of the stent analysed (proximal or distal part) or the protocol of sampling and microbiological analysis [9].

Table 2 Distribution of microbial species isolated in biliary stents

Bacteria	N of stents	
Gram (+)		
Enterococcus spp	38	74%
Streptococcus spp	1	2%
Staphylococcus spp	1	2%
Corynobacterium	1	2%
Gram (-)		
Escherichia coli	32	62%
Klebsiella spp	30	58%
Enterobacter spp	14	27%
Citrobacter spp	8	15%
Pseudomonas spp	8	15%
Morganella morganii	5	10%
Stenotrophomonas maltophilia	3	5%
Aeromonas	3	5%
Proteus spp	1	2%
Hafnia alvey	1	2%
Anaerobes		
Prevotella spp	1	2%
Fungi		
Candida spp	15	29%

Table 3 Microbial species isolated in biliary stents according to disease and stent characteristics

	Malignant vs benign %		Occluded vs non-occluded %		Straight vs pig-tail %		10 Fr vs 7 Fr %	
Enterococcus spp	82	64	80	56	74	75	72	72
Klebsiella spp	47	67	68	37*	54	65	58	59
Pseudomonas spp	21	10	22	0*	22	5	24	4
Candida spp	34	25	40	6*	32	16	34	22
Escherichia coli	43	78*	62	62	51	53	55	72
Enterobacter spp	17	35	22	37	25	20	27	27

*P<0.05, chi square test

Table 4 Isolated bacteria and stent duration in place

	Mean (days)	Median (range) (days)
Enterococcus spp	134.7	94 (9-730)
Klebsiella spp	156.7	116.5 (17-730)
Pseudomonas spp	148	146 (36-255)
Candida spp	177.7	127 (9-730)
Escherichia coli	142.7	98.5 (22-730)
Enterobacter spp	89.5	71 (22-228)
P	NS*	

*Non significant, Mann-Whitney U test

Table 5 Antibiotic resistance of the most frequently isolated organisms

Bacteria	Resistance					
	VRE[a]	ESBLs[b]	AmpC-Dr[c]	MBLs[d]	KPC[e]	QR[f]
Enterococcus spp	1 (2.6%)	-	-	-	-	-
Klebsiella spp	-	1 (3.3%)	-	3 (10%)	1 (3.3%)	6 (20%)
Pseudomonas spp	-	-	-	1 (12.5%)	-	4 (50%)
Escherichia coli	-	4 (12.5%)	-	-	-	11 (34.3%)
Enterobacter spp	-	-	5 (35.7%)	-	-	-

[a]Vancomycin resistant enterococcus, [b]Extended spectrum B-lactamases, [c]AmpC-derepression, [d]Metallo B-lactamases, [e]*Klebsiella pneumoniae* carbapenemase, [f]Quinolone resistant

Another limitation in our study is that patients with symptoms of stent occlusion, and especially cholangitis, received prolonged treatments with antibiotics before stent exchange and that may influence the type of organisms cultured and their sensitivity.

Organisms isolated from the sludge of biliary stents are similar in 47% of patients to those isolated from blood in patients with biliary sepsis [17]. Isolation of similar organisms from blood and from bile shows a wide spectrum from 21-67% of the patients with bacteremia [16,18].

In our study *Enterococcus spp* (74%), *E. coli* (62%), and *Klebsiella spp* (58%) were also the most frequently isolated organisms. Stent diameter (10-Fr vs. 7-Fr) and shape (straight vs. pig-tail) did not play a role in the frequency of organisms. The same bacteria were isolated for benign and malignant disease, with the exception of *E. coli*. *E. coli* was more frequently isolated in benign diseases (P<0.05). Analysis of bile and stent samples of patients with gallstone disease has showed *E. coli* and Gram-negative bacteria to be most commonly found [17,19].

Klebsiella spp, *Pseudomonas spp*, and *Candida spp* were more frequently isolated in occluded than non-occluded stents (P<0.05). This may be due to more hospital admissions and more antibiotics used in patients with occluded stents. *Pseudomonas spp* was not isolated in any non-occluded stent scheduled for extraction.

The incidence of anaerobes in our study was very low (2%). Isolated anaerobic bacteria rates in the literature are controversial because of the difficulties of isolation and proliferation style of some facultative-anaerobic organisms [9,20,21]. However, anaerobic bacteria are suggested to play a significant role in biliary stent clogging [22] and anaerobic therapy is suggested if a biliary-enteric anastomosis is present [23].

The clinical presentation of cholangitis associated with stent occlusion ranges from mild abdominal discomfort and pyrexia to life-threatening septic shock. Antimicrobial therapy is usually empirical. Initial therapy should cover the *Enterobacteriaceae*, especially *E. coli* and relief of biliary obstruction is mandatory. Based on pharmacokinetic studies and *in vitro* susceptibility findings, ciprofloxacin was suggested to be superior to other antibiotics in prophylaxis and treatment of biliary sepsis [24,18]. In our study, *E. coli*, *Klebsiella spp,* and *Pseudomonas spp* were found to be resistant to quinolones in 34%, 20%, and 50% respectively. There was no difference in *E. coli* resistance between occluded and non-occluded stents (P>0.05). Therefore, quinolones may not be adequate either for prophylaxis in patients scheduled for stent exchange, or for treatment of cholangitis associated with stent occlusion. In Europe, the quinolone resistance rate, for hospitalized patients, ranges from 6% (France) to 20% (Spain) [25]. A recent paper demonstrated that *E. coli* resistance to quinolones increased significantly (P<0.0005) between 2007 (20.0%) and 2011 (29.2%) in Canadian hospitals [26]. The increased and often unjustified use of quinolones in the community and in hospitalized patients may have contributed to the high resistance rate in our sample.

Interpreting our results (Table 5) we could suggest that antibiotics combined with ESBLs inhibitors like tazobactam, clavulanate or sulbactam might be the first option in patients with cholangitis associated with stent occlusion, which is in agreement with recent literature [23].

Enterococcus species were the most frequently isolated organisms (74%), but their pathogenicity in biliary tract

Summary Box

What is already known:

- The main drawback of biliary stents is occlusion, usually clinically manifesting as cholangitis
- Bacterial colonization of stents and subsequent biofilm formation are considered as the primary occlusion mechanisms
- Intestinal flora usually colonizes stents by ascending access to the biliary tract

What the new findings are:

- *Enterococcus* species, *Escherichia coli*, and *Klebsiella* species are the most frequently isolated organisms in plastic biliary stents
- In occluded stents *Pseudomonas* species and *Candida* species should be taken into account
- A high resistance to quinolones has been developed

infections remains unclear [17,27]. The need for antibiotic covering against *Enterococcus* should be limited to immunocompromised patients with symptoms of stent occlusion and those in severe sepsis [28].

Candida species were isolated in 40% of occluded stents. Our results are consistent with Negm *et al* [15], where a 31% rate of candida infection in aspirated bile, was found in patients with biliary stents. Fungal infection of the biliary tract is difficult to diagnose and the isolation of fungi may represent only colonisation due to contamination or selection of patients [29]. In patients with reccurent cholangitis or sepsis associated with stent occlusion and malignant disease under chemotherapy, fungal infection has to be taken into account, when designing anti-infectious treatment.

In conclusion, *Enterococcus* species, *E. coli*, and *Klebsiella* species are the most frequently isolated organisms in plastic biliary stents. In occluded stents *Pseudomonas* species and *Candida* species should be taken into account. Quinolones may not to be adequate for the treatment of cholangitis associated with stent occlusion. In patients under chemotherapy for malignancy and stent occlusion related biliary sepsis, antifungal and enterococcal covering should be considered.

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