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Multidrug resistance of isolated microorganisms in occluded bile duct stents*

Tıkanmış bilier stentlerden izole edilen mikroorganizmalardaki çoğul ilaç direnci

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Background/aim: *Biliary stents have been used for benign or malignant biliary strictures, preoperative biliary drainage, resolution of biliary or pancreatic leaks, and dissolution treatment of non-extractable bile duct stones since 1979, and should be replaced at 3-6 month intervals. The aim of this cross-sectional pioneer study was to identify the microorganisms in occluded bile duct stents and to determine incidence and diagnosis of multidrug resistance of the isolated microorganisms in stents and in blood cultures.* ***Methods:*** *Fifty-one patients (14F, 37M, mean age: 58.2±11.6 yr) with cholangitis due to occluded stents were studied consecutively and prospectively after stent replacement was done. Independent variables were age, gender, underlying disease, duration of disease, size, length and period of stents, and blood biochemistry. Dependent variables were microorganisms isolated from the stent content and blood of the patients and the results of antibiogram tests.* ***Results:*** *Primary disease was malignant in 25 (49.0%), stent size was 7F in 30 (58.8%), and stent length was 11 cm in 11 (21.6%) and 15 cm in 12 (23.5%) of the patients. The mean period after the 1st stent application was 207.0±111.3 days and the last stent duration was 111.0±64.0 days. Isolated microorganisms from stents and blood, respectively, were Escherichia coli (E. coli) (43.4%, 20.8%), Klebsiella spp. (17.1%, 17.0%), Pseudomonas aeruginosa (P. aeruginosa) (13.2%, 18.9%) and Enterococcus spp. (10.5%, 17.0%). Isolated microorganisms showed multidrug resistance at high percentages (81.6% for stent, 81.1% for blood). ALT, AST, ALP, and direct bilirubin levels showed statistically significant differences between the benign and malignant groups.* ***Conclusion:*** *All bile duct stents are contaminated by microorganisms, of which a high majority has multidrug resistance, and they frequently cause biliary sepsis. Biliary sepsis can be prevented by changing the stents periodically and by using proper antibiotic prophylaxis.*

Key words: Occluded biliary stents, microorganisms, biliary sepsis, multidrug resistance

Amaç: *Bilier stentler benign ve malign safra yolu darlığı, cerrahi öncesi bilier drenaj gereksinimi, bilier veya pankreatik kanal kaçakları, çıkarılamayan safra yolu taşları bulunan, tedavi veya palyasyon amacıyla ve 3-6 aylık aralıklarla değiştirilmelidir. Bu kesitsel çalışmanın amacı, tıkalı bilier stenti bulunan olgularda alınan stent ve kan kültürleri ile saptanan mikroorganizmaların görülme sıklığı ve çoğul ilaç direnci gösterip göstermediğini araştırmaktır.* ***Yöntem:*** *Stent tıkanmasına bağlı kolanjiti olan, ardışık (14K, 37E, Yaş: 58.2+11.6) 51 olgu, stent değişiminden sonra prospektif olarak incelendi. Bağımsız değişkenler, yaş, cinsiyet, altta yatan hastalık, hastalık ve son stent süresi, çıkarılan stentin çap ve uzunluğu ve kan biyokimyası; bağımlı değişkenler tıkalı stent içeriği ve kan kültürlerinden izole edilen mikroorganizmalar ve antibiyogram sonuçlarıdır.* ***Bulgular:*** *Olguların 25'inde (%49.0) primer hastalık malign, 30'unda (%58.8) stent çapı 7F, stent uzunluğu 11 olguda (%21.6) 11 cm, 12 olguda (%23.5) 15 cm; ilk stent takılmasından itibaren geçen toplam süre 207.0+111.3 gün; son stent kalış süresi 111.0+64.0 gün idi. En çok izole edilen mikroorganizmalar sırasıyla stent ve kan kültürleri için, E.coli (%43.4 ve %20.8), Klebsiella spp. (%17.1 ve % 17.0), P. aeruginosa (%13.2 ve %18.9) ve Enterococcus spp.(%10.5 ve %17.0) olarak tanımlandı. İzole edilen mikroorganizmalar yüksek oranda (Stent kültüründen izole edilen mikroorganizmalarda %81.6, kan kültüründen izole edilen mikroorganizmalarda %81.1) çoğul ilaç direnci gösterdi.* ***Sonuç:*** *Bütün bilier stentler çoğul ilaç direnci gösteren mikroorganizmalarla kontamine olarak tıkanır ve sıklıkla bilier sepsise yol açar. Bilier sepsis, stentlerin periodik olarak değiştirilmesi ve uygun antibiyotik proflaksisi ile önlenmelidir.*

Anahtar kelimeler: Tıkanmış bilier stentler, mikroorganizmalar, bilier sepsis, çoğul ilaç direnci

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INTRODUCTION

Cholangitis is a potentially life-threatening disease that results from bacterial infection of an obstructed bile duct. Systemic toxicity occurs when the intraductal pressure is sufficiently elevated to cause reflux of the bacteria or endotoxin into the blood. Thus, obstruction plays a key role by both increasing intraductal pressure and promoting bacterial overgrowth as a result of bile stasis (1). The options for bile duct decompression include surgical, percutaneous, and endoscopic methods. According to prospective randomized studies, stenting via endoscopic retrograde cholangiopancreatography (ERCP) is better than percutaneous or operative methods due to its several advantages (2). Endoscopic intervention is now accepted as a definitive therapy for acute cholangitis. The advantages of ERCP are that it can delineate the cause of obstruction, facilitate sampling of bile for cultures, and decompress the biliary tree in a relatively short time with low morbidity. Biliary decompression is the goal of therapy and can supply complete opening (e.g., stone removal) or temporary (e.g., placement of a stent without stone removal), needing more definitive management (this will allow stabilization of an unstable patient). Results of the endoscopic treatment with stenting for benign biliary strictures are comparable with its morbidity, stricture rates and survival time (3).

Because stent occlusion can cause a significant late complication, prophylactic replacement has been suggested, and the time interval for stent replacement for malignant biliary tract obstruction has been clarified as 3-6 months, with 4.2% and 10.8% occlusion rates at 3 and 6 months, respectively (4). Acute suppurative cholangitis occurs after the occlusion of stents, and stent replacement or urgent biliary drainage is essential (5).

There are a lot of studies on biliary stents, cholangitis, microbiologic evaluation of infected bile, mechanisms of resistance, glycoprotein mucin structure in endoprosthesis sludge, the phases of the clogging process, deposition of crystals, composition of microbial biofilm (6-12), and the secretory immunoglobulin (13), whereas to date there has been no published study on multidrug resistance (MDR) of isolated bacteria from the occluded stent contents and/or blood in patients with acute suppurative cholangitis. The aim of this cross-sectional study was to evaluate the spectrum of antibiotic susceptibility and MDR of isolated microorganisms both in occluded stents and in blood of the patients with acute suppurative colangitis during stent replacement by ERCP.

MATERIALS AND METHODS

After obtaining informed consent, 51 consecutive patients with occluded biliary stents were included in this prospective study. The major symptoms of the patients were fever (≥38°C), leukocytosis (≥10,000/mm^3), jaundice and abdominal pain. The color of urine, colorless feces, underlying disease, and the type, size, length, date and period of stent were recorded. After physical examination, routine blood analyses for erythrocyte sedimentation rate (ESR), hemoglobin, hematocrit, hemostasis, urinalysis, liver function tests and abdominal ultrasonography (USG) were completed for all patients.

The diagnosis of occluded stent was clarified using dilated intrahepatic bile ducts by ultrasonography, by elevated white blood cell count (WBC), liver enzymes (alanine aminotransferase-ALT, aspartate aminotransferase-AST, alkaline phosphatase-ALP, gamma glutamyl transferase-GGT) and direct bilirubin in blood. All patients were hospitalized, hydrated, fasted and prepared for the ERCP procedure and treated by antimicrobial treatment with quinolone+metronidazole or cefoperazone-sulbactam. After the premedication and sedation using hyoscine-N-butyl-bromide 20 mg and pethidine HCl 50 mg, the occluded stent was removed endoscopically by snare via the 4.2 mm channel of endoscope.

Stents were cut using a sterile blade, and then clogged fragments were taken from the lumina into microcentrifuge tubes containing brain-heart infusion broth, mixed by vortex, and 0.1 ml of each mixture was dropped into blood agar, chocolate blood agar, and MacConkey agar consecutively. Three different blood samples (each 8-10 ml and one taken during stent replacement) were taken from the antecubital vein into BACTEC ™ PLUS (Aerobic/F Blood Culturemedia Benix Limited, Ireland) for the isolation of the microorganisms. After incubation at 37°C for 24-48 hours, the isolated microorganisms were evaluated for identification and for susceptibility to proper antibiotics (14).

The activities of antimicrobial agents in groups were tested against all strains related to being a Gram-positive cocci or Gram-negative bacilli, according to disk diffusion test and minimum inhibitory concentration (MIC) antibiotic sensitivity method. Nine groups of antibiotics for Gram-nega-

tive isolates were classified as 1: aminoglycosides (amikacin, gentamicin, tobramycin), 2: carbapenems (imipenem, meropenem), 3: cephalosporins (1st generation: cefazolin; 2nd generation: cefuroxime, 3rd generation: ceftazidime, cefotaxime, ceftriaxone, cefoperazone, 4th generation: cefepime), 4: aztreonam, 5: aminopenicillins (ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate), 6: antipseudomonal penicillins (piperacillin, piperacillin-tazobactam), 7: cefoperazone-sulbactam, 8: trimethoprim-sulfamethoxazole, and 9: fluoroquinolones: (ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin). For Gram-positive isolates 1: antistaphylococcal penicillins (oxacillin), 2: glycopeptides (vancomycin and teicoplanin), 3: clindamycin, 4: chloramphenicol, 5: tetracyclines, 6: fusidic acid, 7: macrolides (erythromycin, azithromycin, clarithromycin), and 8: rifampin were also added. Furthermore, MIC antibiotic sensitivity was performed by microbroth dilution test using Isosensititest broth (Oxoid™) and MIC system (Phoenix Becton Dickinson and Co. Ireland Benex Ltd. USA). Over 3-5 doubling dilutions that represent a range of concentrations (in µg/ml) at which susceptible bacteria are either inhibited or killed and can be achieved in serum following a standard dose were used. Disk diffusion test results and MIC testing guides antibiotic selection by determining whether the tested bacteria are likely to be sensitive or resistant to any selected antibiotic (14).

The independent variables of the study were age, gender, underlying disease, period of the disease, duration, size and length of the last stent, and liver function tests. Dependent variables were isolated microorganisms, susceptibility to antibiotics and MDR.

There are a lot of MDR definitions for different kinds of bacteria. Most studies are related to molecular or genetic mechanisms of resistance, whereas MDR itself is not yet standardized for each strain. In this study, we have accepted MDR as resistance to two or more drug groups for each isolate (15).

Data was coded and recorded in SPSS for Windows 10.0.1 program. Chi-square, Fisher's exact, Student's t, and Mann-Whitney U tests were used to compare the groups. $P < 0.05$ was accepted as a statistically significant difference.

RESULTS

The mean age of 51 patients (14F, 37M) was 58.2±11.6 (range 39-79) years. Underlying diseases were benign in 26 (51.0%) and malignant in 25 (49.0%) patients. The demographic data, period after first stenting, duration of last stent, stent size and length, number of stent sessions, and distribution of mixed infections according to groups are listed in Table 1. There were no statistically significant differences between benign and malig-

Table 1. Patients and stent characteristics according to groups

		Benign (n = 26)	Malignant (n = 25)	P
Gender	F/M	8/18	6/19	0.588
Age	Mean±SD	59.1±14.1	57.4±8.4	0.637
(Range)		(39-79)	(45-73)	
Period after first stenting		210.5±98.4 days	203.4±125.3 days	0.623
Period of last stenting		107.7±61.6 days	89.0±45.3 days	0.336
Stent Size	7F/10F	16/10	12/13	0.331
Stent length	9 cm	5	-	
	11 cm	7	8	
	12 cm	3	2	
	13 cm	3	3	0.160
	14 cm	3	6	
	15 cm	3	6	
	16 cm	2	-	
Stent sessions	1	12	12	
	2	8	12	
	3	3	1	0.310
	>4	3	-	
Mixed infections	in stent	10	8	0.616
	in blood	3	1	

nant groups according to gender, stent size and length distributions, means of age, period after first stenting, duration of last stent, number of stent replacement sessions, and distribution of mixed infections (P values: 0.588, 0.331, 0.160, 0.637, 0.623, 0.336, 0.310, 0.616, respectively).

The ALT, AST, ALP and direct bilirubin levels were higher in the patients with malignant diseases, with statistically significant differences (P: 0.002, 0.031, 0.010, 0.000, respectively), but there was no significant difference in GGT levels between benign and malignant cases (Table 2).

Table 2. Liver function tests according to groups (Mean ± SD)

Tests	Benign	Malignant	P
ALT U/L	42.5±19.7	62.6±42.6	0.002
AST U/L	59.4±41.5	60.9±61.7	0.031
ALP U/L	845.6±584.0	1023.5±905.7	0.010
GGT U/L	255.8±207.0	363.1±282.8	0.199
D. Bilirubin mg/dl	2.9±3.6	5.7±8.8	0.000

Bacteriologic Findings

The percentages of positive culture were 100% (51 cases) in stent and 86.3% (44 cases) in blood culture. A total of 76 species of bacteria were isolated from the stents of 51 patients and 53 species from blood cultures of 44 patients. Two different species in 15 (29.4%) stents and in 2 (4.5%) blood, and 3 different species in 4 (7.8%) stents and in 2 (4.5%) blood cultures were isolated. The frequency of microorganisms isolated from bile and blood cultures are shown in Tables 3 and 4. The most frequently isolated microorganisms from stents and from blood cultures, respectively were *Escherichia coli* (*E. coli*) (43.4%, 20.8%), *Klebsiella spp*. (17.1%, 17.0%), and *Pseudomonas aeruginosa* (*P. aeruginosa*) (13.2%, 18.9%). There were no statistically significant differences in the incidence of different microorganisms between benign and malignant cases.

Table 3. Isolated microorganisms from stents according to underlying disease

Microorganisms	Benign	Malignant	Total
C. albicans	1 (2.9%)	1 (2.4%)	2 (2.6%)
Klebsiella spp.	8 (23.5%)	5 (11.9%)	13 (17.1%)
MR S. aureus	1 (2.9%)	4 (9.5%)	5 (6.6%)
P. aeruginosa	2 (5.9%)	8 (19.0%)	10 (13.2%)
S. aureus	2 (5.9%)	3 (7.1%)	5 (6.6%)
E. coli	13 (38.2%)	20 (47.6%)	33 (43.4%)
Enterococcus spp.	7 (20.6%)	1 (2.4%)	8 (10.5%)
Total	34 (44.7%)	42 (55.3%)	76 (100.0%)

X^2: 11.563, P: 0.072 > 0.05

Table 4. Isolated microorganisms from blood according to underlying disease

Microorganism	Benign	Malignant	Total
Acinetobacter spp.	2 (7.6%)	1 (3.7%)	3 (5.7%)
C. albicans	3 (11.5%)	-	3 (5.7%)
Klebsiella spp.	6 (23.1%)	3 (11.1%)	9 (17.0%)
MR S. aureus	4 (15.4%)	2 (7.4%)	6 (11.3%)
P. aeruginosa	5 (19.2%)	5 (18.5%)	10 (18.9%)
S. aureus	-	2 (7.4%)	2 (3.8%)
E. coli	4 (15.4%)	7 (25.9%)	11 (20.8%)
Enterococcus spp.	2 (7.6%)	7 (25.9%)	9 (17.0%)
Total	26 (44.7%)	27 (55.3%)	53 (100.0%)

X^2: 12.017, P: 0.150 > 0.05

Table 5 shows the resistance percentages of antibiotics, the overall resistance ratio, and MDR ratios of 76 microorganisms isolated from the stents. According to this table, microorganisms show high percentage (81.6%) of total MDR, except for *Candida albicans (C. albicans)* and *Enterococcus spp.* There were no isolated *Acinetobacter* strains from occluded bile stents.

Resistance ratios of antibiotics against 53 isolates of microorganisms from blood are listed in Table 6. The most resistant species were *Acinetobacter spp*., *methicillin-resistant Staphylococcus aureus* (*S. aureus*), *P. aeruginosa*, *E. coli* and *Klebsiella spp*., whereas there was no resistance of *C. albicans*, and very low resistance ratio of *Enterococcus spp.* MDR ratios were higher for each antibiotic in microorganisms from blood cultures than those from occluded stent contents, and total MDR ratio was calculated as 81.1%. There was no resistant microorganism against glycopeptides and fluconazole.

DISCUSSION

In the study, we found *E. coli* (43.4%), *Klebsiella spp.* (17.1%), and *P. aeruginosa* (13.2%) in occluded stents, and *E. coli* (20.8%), *P. aeruginosa* (18.9%), *Klebsiella spp.* (17.0%) and *Enterococcus spp.* (17.0%) in blood cultures; there was no *Streptococcus*, *Bacteroides*, or *Clostridium* species.

Table 5. Resistance ratios of antibiotics against 76 isolates of microorganisms from bile

Antibiotics	Candida alb.	Klebsiella spp.	M. Resist. S. aureus	Pseud. aeruginosa	Staph. aureus	Esch. coli	Enterococcu spp.	Total
Aminoglycosides	-	15.4	-	20.0	-	21.2	12.5	23.4
Carbapenems	-	7.7	-	40.0	-	9.1	12.5	15.6
3rd&4th generation cephalosporins	-	46.2	-	90.0	-	36.4	-	43.8
Aztreonam	-	30.8	-	80.0	-	48.5	12.5	45.3
Aminopenicillins	-	61.5	100.0	-	0.0	24.2	12.5	34.4
Anti-Pseudo. Pen.	-	69.2	-	80.0	-	60.6	12.5	50.0
Cefoperazone-Sulbactam	-	30.8	-	80.8	-	33.3	-	37.5
Trimethoprim + Sulfamethoxazole	-	61.5	60.0	-	40.0	36.4	12.5	40.6
Fluoroquinolones	-	46.2	-	50.0	-	48.5	12.5	43.8
Anti-Staph. Pen.	-	-	100.0	-	0.0	-	-	50.0
Glycopeptides	-	-	0.0	-	0.0	-	-	0.0
Clindamycin	-	-	100.0	-	20.0	-	-	60.0
Chloramphenicol	-	-	20.0	-	0.0	-	-	10.0
Tetracyclines	-	-	100.0	-	60.0	-	-	80.0
Fusidic Acid	-	-	40.0	-	0.0	-	-	20.0
Macrolides	-	-	80.0	-	40.0	-	-	60.0
Rifampin	-	-	80.0	-	60.0	-	-	70.0
Fluconazole	0.0	-	-	-	-	-	-	0.0
MDR	0.0	92.3	100.0	100.0	60.0	93.9	12.5	81.6

Table 6. Resistance ratios of antibiotics against 53 isolates of microorganisms from blood

Antibiotics	Cinetobat. spp.	Candida alb.	Klebsiella. spp.	M. Resis. S. aureus	Pseud. aureginosa	Staph. aureus	Esch. coli	Enterocacus spp.	Total
Aminoglycosides	66.7	-	11.1	-	20.0	-	27.3	11.1	21.4
Carbapenems	100.0	-	0.0	-	40.0	-	9.1	11.1	21.4
3rd&4th generation cephalosporins	100.0	-	55.6	-	90.0	-	36.4	-	52.4
Aztreonam	100.0	-	44.4	-	80.0	-	54.5	11.1	52.4
Aminopenicillins	100.0	-	66.7	100.0	-	100.0	27.3	11.1	40.6
Anti-Pseudo. Pen.	100.0	-	66.7	-	80.0	-	63.6	11.1	59.5
Cefoperazone-Sulbactam	66.7	-	33.3	-	80.8	-	36.4	-	42.9
Trimethoprim + Sulfamethoxazole	100.0	-	55.6	95.5	-	-	45.5	11.1	43.8
Fluoroquinolones	66.7	-	55.6	-	50.0	-	45.5	11.1	42.9
Anti-Staph. Pen.	-	-	-	100.0	-	0.0	-	-	75.0
Glycopeptides	-	-	-	0.0	-	0.0	-	-	0.0
Clindamycin	-	-	-	100.0	-	50.0	-	-	87.5
Chloramphenicol	-	-	-	16.7	-	50.0	-	-	25.0
Tetracyclines	-	-	-	83.3	-	50.0	-	-	75.0
Fusidic Acid	-	-	-	33.2	-	0.0	-	-	25.0
Macrolides	-	-	-	50.0	-	50.0	-	-	50.0
Rifampin	-	-	-	66.7	-	50.0	-	-	62.5
Fluconazole	-	0.0	-	-	-	-	-	-	0.0
MDR	100.0	0.0	100.0	100.0	100.0	100.0	100.0	22.2	81.1

Analysis of bile and stone cultures indicates that *E. coli*, *Klebsiella spp.*, *Enterobacter spp*., *Enterococcus spp.*, and *Streptococcus spp.* are the most commonly isolated bacteria (16). For gallstone diseases, the most common organisms cultured were Gram-negative bacteria species (74%), as *E. coli* (36%) and *Klebsiella* (15%) were most commonly found, followed by Gram-positive (15%) bacteria such as *Enterococcus* (6%), *Staphylococcus* (3%), and *Streptococcus* (2%). *Bacteroides* (5%) and *Clostridium* (3%) species were occasionally found anaerobes (9%) (17). With respect to aerobic microorganisms, our results were similar to the literature.

In contrast with the literature, we found polymicrobial infection ratios to be 11.8% from occluded stents and 7.5% from blood. In the literature, polymicrobial infection percentage was reported in 19% for patients with gallbladder stones, 31% for common bile duct stones and 29% for intrahepatic duct stones, and frequency of mixed aerobic+anaerobic infection was 7%, 12% and 9%, respectively (17). The incidence of isolated anaerobic strains is heterogeneous and controversial in the literature. We believe that isolated anaerobic microorganism ratios in the literature are questionable because of the difficulties of isolation and proliferation style of some facultative-anaerobic microorganisms.

Isolation of similar microorganisms in bile duct stents and in blood is important for the diagnosis and treatment of biliary sepsis. We found similar organisms from the occluded stents and from the blood at a rate of 47.1% out of 51 patients, which is parallel to the literature. Isolation of similar organisms from blood and from bile shows a wide spectrum from 21% to two-thirds (67%) of the patients with bacteremia (17, 18).

Occlusion of stents leads to progressive extinction of the biofilm and mummification of its components. Deposition of cholesterol or other substances within the biofilm matrix may be a novel mechanism of host defense against bacteria present in these biofilms (11). Some bacteria causing several kinds of human infectious diseases are resistant to multiple antibiotics and are continuing to increase (15). Resistant infections confront and thwart the treatment of some patients in the community as well as in the hospital. Major resistant hospital organisms include *S. aureus*, *Enterococcus*, *Klebsiella, Enterobacter, E. coli*, *Pseudomonas* and more recently *Acinetobacter*. MDR bacteria causing community-acquired infections include *pneumococcus*, *gonococcus*, *Mycobacterium tuberculosis*, group A *streptococci* and *E. coli*.

In the present study, 34.4% of microorganisms isolated from occluded bile stents and 40.6% of microorganisms isolated from blood were found to be resistant to ampicillin-sulbactam. In one study, ampicillin in combination with sulbactam and aminoglycoside was accepted as suggestive empirical therapy, and it was suggested that antibiotic treatment should be adjusted based on later bacteriological cultures and clinical condition (17). Our findings have suggested that cefoperazone-sulbactam, aminoglycosides and quinolones are similar or better than ampicillin-sulbactam for empiric treatment until obtaining results of cultures from the patients with replacement of occluded stent.

Pharmacokinetic studies on the hepatic/biliary excretion profiles of ceftazidime, cefoperazone, imipenem, netilmicin, and ciprofloxacin were performed by ERCP and nasobiliary catheter drainage. The bile samples obtained immediately after cannulation from patients with complete biliary obstruction contained low or undetectable levels of the antibiotics administered - the exception being ciprofloxacin, which was present at a concentration of 20% of the serum level at the same time. In vitro determination of MIC of the aforementioned antibiotics against isolates of biliary pathogens revealed imipenem and ciprofloxacin to have the highest antimicrobial activity. Based on pharmacokinetic studies and in vitro susceptibility findings, it was concluded that ciprofloxacin was superior to the other tested antibiotics in prophylaxis and treatment of biliary sepsis (18).

In the present study, *E. coli* strains showed from 9.1% to 63.6% resistance; *Enterococcus spp.* strains were mostly sensitive to all appropriate antibiotics, and resistance rate was calculated as 11.1%. Overall multiple antibiotic resistance rates (resistance against 2 or more groups of antibiotics) were calculated as 81.6% for microorganisms isolated from occluded stents and 81.1% for microorganisms isolated from blood. According to a study, *Enterobacteriaceae* are sensitive to netilmicin (100%), imipenem (98.1%), ciprofloxacin (96.3%), cefotaxime (68.5%), and piperacillin (56.9%), whereas *Enterococcus spp.* were sensitive to imipenem (79%), piperacillin (75%), ciprofloxacin (63%), and ampicillin (58%); high rates of antibiotic resistance suggest that bacteriologic monitoring is mandatory to avoid treatment failures in patients with occluded biliary stents (18, 19).

Management of patients with occluded stents is important. Factors affecting the efficacy of antibacterial therapy include the activity of the agent against the common biliary tract pathogens and pharmacokinetic properties such as tissue distribution and the ratio of concentration in both bile and serum to the MIC for the expected microorganism. Antimicrobial therapy is usually empirical. Initial therapy should cover the Enterobacteriaceae, in particular *E. coli*. Activity against *Enterococci* is not required since their pathogenicity in biliary tract infections remains unclear. Coverage of anaerobes, in particular *Bacteroides spp*., is warranted in patients with previous bile duct-bowel anastomosis, in the elderly and in patients in serious clinical condition. In patients with acute cholecystitis or cholangitis of moderate clinical severity, monotherapy with a ureidopenicillin - mezlocillin or piperacillin - is at least as effective as the combination of ampicillin plus aminoglycoside. In severely ill patients with septicemia, an antibacterial combination is preferable. Therapy with aminoglycosides, mostly for *P. aeruginosa*-related infections, should not exceed a few days because the risk of nephrotoxicity seems to be increased during cholestasis. Relief of biliary obstruction is mandatory, even if there is clinical improvement with conservative therapy, because cholangitis is most likely to recur with continued obstruction. Emergency invasive therapy is reserved for patients who fail to show a clinical response to antibacterial therapy within the first 36 to 48 hours or for those who deteriorate after an initial clinical improvement. Antibacterial prophylaxis before ERCP and long–term administration of proper antibacterials are required for prolonged jaundice and recurrent cholangitis after wound infections after biliary tract surgery and biliary-enteric anastomoses. For these indications, piperacillin, cefazolin, cefuroxime, cefotaxime, cefoperazone+sulbactam and ciprofloxacin are effective because they can be excreted into bile in the patients with biliary obstruction (20).

Mortality and hospital length of stay were prolonged for resistant strains of some organisms compared with susceptible ones. In conclusion, according to the literature, treatment and research priorities must be: 1- The replacement of stents before occlusion in periods of 3-6 months, 2- Early diagnosis, isolation and treatment of the microorganisms in the patients with biliary sepsis, 3- Detection of molecular mechanisms of resistance, 4- Development of surveillance systems using practitioners and hospital and private laboratories, detection of new resistance mechanisms, and detection of development of resistance in normally susceptible organisms; plus, according to our study, 5- Development of drugs to deal with newly-resistant organisms; and 6- Enhancement of public and professional awareness that the antimicrobial treatment problem is real.

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