CORRESPONDENCE

Comment on Demirbağ, et al. "Multidrug resistance of isolated microorganisms in occluded bile duct stents" (Turk J Gastroenterol 2007; 18 (1): 33-40)

To the Editor

I have read, with great interest, the article by Demirbağ et al. (1) published in Turk J Gastroenterol which showed that all bile duct stents are contaminated by microorganisms, the majority of them being multidrug resistant. However, I have some comments to this article.

First, the resistance rate of Klebsiella to aminopenicillin is shown in Table 5 and Table 6 of this article (61.5%), but it is very well known that Klebsiella spp have innate resistance to ampicillin and amoxicillin (2). It is unnecessary to include these antibiotics in in vitro susceptibility tests for *Klebsiella spp*. Furthermore, even if these tests are performed and found to be sensitive, they should be reported as resistant (3) because this resistance expresses itself in in vivo conditions; it cannot express itself resistant in in vitro conditions (4). Similarly, the resistance rate for *Enterococcus* to aztreonam, trimethoprim sulfamethoxazole and aminoglycosides seems to be 12.5% in bile and 11.1% in blood culture, which indicate 87.5% and 89.9% sensitivity, respectively, which is impossible, since enterococci also have innate resistance to aztreonam, trimethoprim sulfamethoxazole, and aminoglycosides (4). Additionally, aminoglycosides are used for synergistic effect in the treatment of enterococcus, and not used alone. This issue should be clarified.

Secondly, the way of measuring the methicillin resistance to *Staphylococcus aureus* in the methods section was not described. It would be better if the authors had described the method. Other than this, the rate of resistance for MRSA to fucidic acid reported in this article (40%) seems to be very high, since it has been reported to be between 0% and 19.4% in many studies (5-7). Do the authors have any comments regarding the possible reason for this high resistance?

Thirdly, the rate of resistance to third and fourth generation cephalosporins for *Pseudomonas aeruginosa* has been reported to be 90% in this article. Which antibiotic does this resistance stand for - ceftazidime, ceftriaxone or cefotaxime? This rate is also very high, and should be explained.

Lastly, if an erratum is going to be published, it should also include correction of spelling errors in the first line of Table 6 (names of bacteria have been written as "Cinetobat. spp." and "Enterocacus spp.").

REFERENCES

1. Demirbağ AE, Karademir A, Parlak E, et al. Multidrug resistance of isolated microorganisms in occluded bile duct stents. Turk J Gastroenterol 2007; 18: 33-40.
2. Chambers HF. Penicillins. In: Mandell GL, Bennet JE, Dolin R, eds. Principles and practice of infectious diseases. 6th ed. Churchill Livingstone 2005; 281-94.
3. Bal Ç. Etkene yönelik tedavide antibiyogram yorumu. Türkiye Klinikleri J Int Med Sci 2005; 1: 39-49.
4. Livermore DM, Winstanley TG, Shannon KP. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. J Antimicrob Chemother 2001; 48: 87-102.

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5. Randrianirina F, Soares JL, Ratsima E, et al. In vitro activities of 18 antimicrobial agents against Staphylococcus aureus isolates from the Institut Pasteur of Madagascar. Ann Clin Microbiol Antimicrob 2007; 6: 5.
6. Baddour MM, Abuelkheir MM, Fatani AJ. Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. Ann Clin Microbiol Antimicrob 2006; 5: 30.
7. Shittu AO, Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates of Staphylococcus aureus in KwaZulu-Natal province, South Africa. BMC Infect Dis 2006; 6: 125.

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REPLY

First of all we would like to express our heartfelt thanks for the author's attention, kind sentences, questions, comments and corrections.

The aim of this pioneer study was to identify resistance ratios in microorganisms isolated from occluded stents and blood that were higher than those expected, and declare the multi-drug resistance. All data was taken from the antibiotic susceptibility tests under laboratory conditions, but all of the patients were treated using proper antibiotics, and there was no complication or untreated patient and mortality.

Our hospital is a post-graduate, large volume, tertiary teaching and research hospital. Many complicated primary or referred patients with gastrointestinal cancers, biliary-enteric fistulae, and biliary strictures, and patients previously operated/instrumented/stented several times sent from primary and secondary hospitals are treated in our Gastroenterology and Gastrointestinal Surgery clinics and intensive care units (ICUs). In addition, the mean period of hospitalization in the ICU for patients is relatively higher than for those in primary and secondary hospitals. As a result of these features, we have to use antibiotics very often according to medical indications. More antibiotic usage means greater resistance ratio of antibiotics in the hospital.

We have an infection control committee and an active infection surveillance system. Antibiotic usage and indications have been strictly controlled by this committee since the 1980s. Only prophylactic antibiotic usage (mostly 1st generation cephalosporins) is managed by the clinician without referring to the committee. For the prevention and management of nosocomial infection, all patients who undergo elective surgery or instrumentation and are candidates for hospitalization in ICUs are controlled by nose and throat cultures, and positive cases are treated before hospitalization.

Answers to specific questions:

1. We isolated *Klebsiella* species in 13 stents and 9 blood samples as shown in Tables 3 and 4, and none of them was extended spectrum ß-lactamase (*ESBL*). Aminopenicillins (with clavulanic acid or sulbactam) may be a treatment alternative and can be used for the treatment of non-*ESBL* strains with more than 60% susceptibility in *Klebsiella* spp (1). We usually prefer aminopenicillins (with clavulanic acid or sulbactam) in only antibiograms, but we use cefoperazone + sulbactam or piperacillin + tazobactam for the treatment of *Klebsiella* spp. in patients with occluded stents.

We isolated *Enterococcus* from 8 stents and 9 blood samples and there was only one resistant strain in each group (Tables 3-6). The row for aztreonam in the *Enterococcus* column should have been left blank in Tables 5 and 6. We would like to herein correct these unintentional errors with our apology and appreciation to the authors. We preferred trimethoprim-sulfamethoxazole and aminoglycosides in the antibiogram tests. In the evaluation of hepatic and renal functions, we prefer aminoglycoside + ampicillin (with sulbactam) (or glycopeptide for hypersensitive patients or ampicillin-resistant strain) for the treatment for *Enterococcus* in patients with occluded stents (1).

2. Methicillin resistance of *Staphylococcus aureus* is measured by oxacillin screening agar method in our hospital. A total of 11 *methicillin-resistant Staphylococcus aureus* (MRSA) (5 from stents, 6 from blood) species were isolated in this study (Tables 3 and 4). We think the conditions of our

Original Versions

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	Enterococcus 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

Revised Versions

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

Antibiotics	*Candida albicans*	*Klebsiella spp.*	*MRSA*	*P. aeruginosa*	*S. aureus*	*Escherichia coli*	*Enterococcus 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&4thgen. cephalosporin	-	46.2	-	90.0	-	36.4	-	43.8
Aztreonam	-	30.8	-	80.0	-	48.5	-	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
TMP+SMX	-	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
Multiple Drug Resistance	0.0	92.3	100.0	100.0	60.0	93.9	12.5	81.6

TMP-SMX: Trimethoprim-sulfamethoxazole, MRSA: Methicillin-resistant *S. aureus*

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

	MICROORGANISMS								
Antibiotics	***Acinetobacter spp.***	***Candida albicans***	***Klebsiella spp.***	***MRSA***	***P.aeruginosa***	***S. aureus***	***Escherichia coli***	***Enterococcus 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 gen. cephalosporin	100.0	-	55.6	-	90.0	-	36.4	-	52.4
Aztreonam	100.0	-	44.4	-	80.0	-	54.5	-	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
TMP+SMX	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

MDR: Multiple drug resistance, TMP-SMX: Trimethoprim-sulfamethoxazole, MRSA: Methicillin-resistant *S. aureus*

hospital, characteristics of our patients as described before and resistance mechanisms of microorganisms may be responsible for the high level (40% of MRSA from stents and 33.2% of MRSA from blood) of fucidic acid resistance. We also think that it is not possible to draw an exact conclusion based on this small number of MRSA species.

3. 3rd and 4th generation cephalosporin (ceftazidime) resistance of 20 *P. aeruginosa* (10 from stents, 10 from blood) species was calculated as 90% for each. Our hospital's infection control committee also reports the same resistance ratios for *P. aeruginosa*. We do not use cefoperazone + sulbactam routinely in antibiogram for *P. aeruginosa*, *E. coli* and *Klebsiella* species. In resistant *Pseudomonas* species, and *ESBL*-positive *E. coli* and *Klebsiella* species, we also prefer cefoperazone + sulbactam disk diffusion test for antibiogram in order to make a treatment alternative.

We would like to stress some information on the bacterial resistance mechanism from the literature that had been included in the manuscript but was thereafter removed according to the reviewer's evaluation of this article:

"Stent occlusion" phenomenon has been partially clarified since the 1980s. The 1990s is the era of multidrug resistance. In an attempt to establish why biliary endoprostheses clog, after the investigation of the contents of occluded endoprostheses, the major components of the endoprosthesis sludge were glycoprotein mucin, an insoluble residue which consisted mainly of plant fibers, bacterial clumps, and bacterial slime. Scanning electron microscopy of the walls of clogged endoprostheses revealed adherence of amorphous material, probably protein. The initial phase in the clogging process is adsorption of proteins, after which other materials such as bacteria, fibers from food and unconjugated bilirubin bind to the wall of the endoprosthesis. The material blocking the lumina was composed of a matrix of bacterial cells and their fibrillar anionic extracellular products. Crystals of calcium bilirubinate, calcium palmitate, and cholesterol were embedded within this matrix. Bacterial cells were attached to the stent surface by a fibrillar matrix, suggesting that the initial event in stent clogging is the development of an adherent bacterial biofilm. Bacterial enzyme activity (beta-glucuronidase and phospholipase) leads to the deposition of crystals (2-4).

Direct observations have clearly shown that biofilm bacteria predominate, numerically and metabolically, in virtually all nutrient-sufficient ecosystems. Biofilm cells are at least 500 times more resistant to antibacterial agents. Each biofilm bacterium lives in a customized microniche in a complex microbial community that has primitive homeostasis, a primitive circulatory system, and metabolic cooperativity, and each of these sessile cells reacts to its special environment (5). Biological proliferation is optimized at various levels of organization, including the molecule (e.g. nucleic acids, prions), the cell (e.g. prokaryotic cells, eukaryotic cells), and the community (e.g. microbial biofilms, bioaggregates) (6).

The central bulk of the stent deposits appears as an amorphous, structureless material. IgA was found as a rim of dark brown discoloration at the periphery. IgG shows similar distribution and intensity to that of IgA, whereas little IgM is detected. Bile immunoglobulins may facilitate bacterial adhesion, clumping, and hence biofilm formation on the stent surface (7). A wide range of different branches and groups of bacteria participate in the development of biofilms on the surfaces of foreign bodies, such as biliary stents, mixed gall stones, or calcific pancreatic ducts, but not on the surface of pure cholesterol gall stones. 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 (8). Some bacteria causing several kinds of human infectious diseases are resistant to multiple antibiotics and are continuing to increase (9).

4. There were no spelling errors in the original manuscript. We think mistakes were made during the publishing process. Corrections: Error 1: *Acinetobacter spp*. instead of *Cinetobat. spp*. Error 2: *Enterococcus spp*. instead of *Enterocacus spp*. in Table 6. [Note: further formatting changes were also made to the Tables by the proofreader].

In conclusion, all of the resistant microorganisms need to be studied separately in order to identify the resistance mechanism at the molecular level.

REFERENCES

1. Gilbert DN, Moellering RC Jr, Eliopoulos GM, Sande MA. The Sanford guide to antimicrobial therapy. 36th ed. Sperryville, VA, USA: Antimicrobial Therapy Inc., 53-5.
2. Groen AK, Out T, Huibregtse K, et al. Characterization of the content of occluded biliary endoprostheses. Endoscopy 1987; 19: 57-9.
3. Zhang H, Tsang TK, Jack CA. Bile glycoprotein mucin in sludge occluding biliary stent. J Lab Clin Med 2003; 142: 58-65.
4. Speer AG, Cotton PB, Rode J, et al. Biliary stent blockage with bacterial biofilm. A light and electron microscopy study. Ann Intern Med 1988; 108: 546-53.
5. Costerton JW, Lewandowski Z, Caldwell DE, et al. Microbial biofilms. Annu Rev Microbiol 1995; 49: 711-45.
6. Caldwell DE. The calculative nature of microbial biofilms and bioaggregates. Int Microbiol 2002; 5: 107-16.
7. Chan FK, Suen M, Li JY, Sung JJ. Bile immunoglobulins and blockage of biliary endoprosthesis: an immunohistochemical study. Biomed Pharmacother 1998; 52: 403-7.
8. Swidsinski A, Schlien P, Pernthaler A, et al. Bacterial biofilm within diseased pancreatic and biliary tracts. Gut 2005; 54: 388-95.
9. Levy SB. Multidrug resistance--a sign of the times. N Engl J Med 1998; 338: 1376-8.

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