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ORIGINAL ARTICLE

Evaluating the frequency and risk factors of multidrug-resistant bacteria in biliary samples

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ABSTRACT

Background and Objectives: This study aimed to evaluate the frequency of multidrug-resistant (MDR) bacteria in biliary samples, MDR-bacteria risk factors, and the relationship between MDR-bacteria positivity and some clinical outcomes.
Materials and Methods: The study was conducted between May 2018 and May 2023, including patients over the age of 18 who had positive culture results in biliary samples. The frequency of MDR-bacteria in biliary samples was evaluated. Risk factors for MDR bacteria were assessed using univariate and multivariate analyses. MDR and non-MDR groups were compared inappropriate empirical antibiotic treatment, total antibiotic treatment duration, length of stay, and in-hospital mortality.
Results: 342 microorganisms were isolated from 202 patients. *Escherichia coli* was the most commonly (37.2%) isolated Gram-negative microorganism, and *Enterococcus* spp. was the most commonly (70.2%) isolated Gram-positive microorganism. The incidence of MDR microorganisms was 42.3%. Gastrointestinal malignancy (OR: 1.96; 95% CI, 1.03-3.71) and previous antibiotic use (OR: 2.26; 95% CI, 1.09-4.68) were independent risk factors for MDR-bacteria. In the MDR group, inappropriate empirical antibiotic treatment (56.6% vs. 41%, p = 0.091), total antibiotic treatment duration (13 vs. 8 days, p = 0.054), length of stay (24 vs. 15 days, p = 0.001), and in-hospital mortality (27.3% vs. 22.3%, p = 0.416) were higher compared to the non-MDR group.
Conclusion: MDR-bacteria positivity is associated with inappropriate antibiotic treatment, prolonged hospitalization, and increased mortality. Screening, antibiotic prophylaxis, and empirical treatment approaches should be carefully performed in patients with malignancy and recent antibiotic use, which are significant risk factors for MDR-bacteria.

Keywords: Gastrointestinal neoplasms; Antimicrobial stewardship; Antibiotic prophylaxis; Biliary tract; Drug resistance; Microbial; Colorectal surgery

INTRODUCTION

Multidrug-resistant (MDR) bacterial infections are a priority due to high attributable mortality, increased healthcare costs, and inadequate infection control (1). Current studies still investigate the effect of infection or colonization with MDR-bacteria in different systems or organs on clinical outcomes.

The biliary system is susceptible to selecting MDR microorganisms due to its interaction with the gut microbiome (2). The prevalence of MDR-bacteria, especially Gram-negative bacteria, in biliary sam-

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ples has increased (3). Since this prevalence changes according to predisposing conditions, it is crucial to identify risk groups for MDR-bacteria related infections. In different studies, aging, immunosuppression, and invasive procedures were some risk factors for MDR-bacteria related biliary system infections (4). Besides biliary tract infection, MDR-colonization in bile is associated with subsequent infections and poor infection-related outcomes (5, 6). There is an association between MDR-bacteria colonization in bile and surgical site infections, bloodstream infections, and shortened survival in patients with gastrointestinal malignancies. However, the published data includes small and different patient groups, such as cancer or post-surgery patients (7, 8). Apart from clinical outcomes, the prevalence of MDR bacteria is crucial for appropriate antibiotic prophylaxis, empirical antibiotic treatment, and effective infection control measures.

This study aimed to evaluate MDR-bacteria prevalence in biliary samples, MDR-bacteria risk factors, and the relationship between MDR-bacteria positivity and some clinical outcomes.

MATERIALS AND METHODS

Study design. This is a database study. The data was collected from the microbiological database of the hospital between May 2018 and May 2023. The study was approved by the Ethical Committee of Gazi University (Date: 28.11.23).

Study population. Patients over 18 years old who have yielded a positive culture result in biliary samples obtained during the operation, endoscopic retrograde cholangiopancreatography (ERCP), or percutaneous transhepatic cholangiography (PTC). Only the first biliary sample was taken into account in repetitive cultures. The patients who had no bacterial growth were excluded from the study.

Study protocol. The data of the patients who met the criteria was obtained from the medical records. Age, gender, accompanying comorbidities, Charlson Comorbidity Index score (CCI), previous surgery, presence of internal/external biliary stents, previous antibiotics, and hospitalization (within the last three months) were recorded. Biliary samples obtained during the operation, ERCP, or PTC transported in sterile containers to a microbiology laboratory for culture. Biliary samples inoculated on 5% sheep blood agar, Eosin Methylene blue (EMB) Agar, and Sabouraud Dextrose Agar (SDA), and incubated at 35-38°C for 24-48 hours. Microorganism identifications were made with MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and phenotypic susceptibility tests were made with VITEK 2 (bioMérieux, Marcy l'Étoile, France). Antibiotic susceptibility results are reported according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints. The microorganisms isolated from biliary samples were classified as multidrug-resistant (MDR) and non-MDR with the results of antibiotic susceptibilities. Both MDR and non-MDR groups were compared for the risk factors of colonization/infection.

Definitions. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (9). Inappropriate use of antibiotics was defined as non-susceptibility to the antibiotics which started empirically. Previous antibiotic use was accepted as the use of antibiotics within the last three months before culture positivity.

Outcomes. The primary outcome of the study was to determine the frequency of MDR microorganisms in biliary samples and to detect the risk factors for their presence. The secondary outcome was to evaluate the effect of MDR positivity on inappropriate antibiotic use, duration of antibiotics, length of stay, and in-hospital mortality.

Statisticsal analyses. The statistical analyses were performed using Statistical Package for Social Sciences (SPSS) v25.0 for Mac OS X (SPSS Inc., Chicago, IL, USA). The normality of the data distribution was determined by the Shapiro–Wilk test, histograms, and Q-Q plots. Categorical variables were expressed as the number of subjects (n) and the percentage value (%), while continuous variables were given as mean (standard deviation) or median (interquartile range). Comparison between groups was performed for non-parametric variables with the Mann-Whitney U test and parametric variables with the Student-t test.

To determine the risk factors affecting the presence of MDR, patients were divided into two groups: patients from whom MDR microorganisms were isolated and patients from whom MDR microorganisms could not be isolated. Variables with a p-value <0.2

and n ≥ 10 (for categorical variables) in the univariate analysis were included in a multivariate regression model using backward-LR. Correlations among these variables were analyzed using Spearman's test. In each pair, the variable that detected a high correlation (rho >0.75) with the other variable was excluded from the regression model. To assess the model's goodness of fit, the Hosmer–Lemeshow test was performed. The 95% confidence intervals (95% CIs) were calculated whenever appropriate, and a two-tailed p-value of <0.05 was considered statistically significant.

RESULTS

A total of 202 patients were included in the study. The clinical characteristics of the patients are summarized in Table 1. The frequency of MDR microorganisms was 42.3% (n=119). CCI, gastrointestinal malignancies, previous antibiotic use, hospitalization within the last three months, and hospital acquired infections were significant risk factors for the presence of MDR microorganisms in univariate analysis (p<0.2). CCI was not included in the model due to its high correlation with gastrointestinal malignancy. In multivariate analysis, gastrointestinal malignancy (OR: 1.96; 95% CI, 1.03-3.71) and previous antibiotic use (OR: 2.26; 95% CI, 1.09-4.68) were found as independent risk factors.

The effect of MDR positivity on the outcomes is presented in Table 2. 161 patients received empirical antibiotherapy. In the MDR group, inappropriate empirical antibiotherapy was 56.6% (n=69), whereas in the non-MDR group, it was 41.0% (n=16), but this difference was not statistically significant (p=0.091). 121 patients received targeted antibiotic therapy. The median duration of antibiotic treatment was longer in the MDR group compared to the non-MDR group, but

Table 1. Demographic and clinical findings of patients

	All of the patients N = 202	MDR N = 99	Non-MDR N = 103	P value	Adjusted OR
Age, median (IQR)	65.0 (56.75-72.25)	65.0 (57.0-73.0)	64 (55.0-72.0)	0.289	
Male gender, N (%)	130 (64.4)	67 (67.7)	63 (61.2)	0.334	
Intensive Care Unit (ICU)	47 (23.3)	19 (19.2)	28 (27.2)	0.179	0.606 (0.30-1.20)
Comorbidities, N (%)					
Hypertension	69 (34.2)	33 (33.3)	36 (35.0)	0.808	
Diabetes mellitus	57 (28.2)	28 (28.3)	29 (28.2)	0.984	
Gastrointestinal malignancy**	145 (71.8)	77 (77.8)	68 (66.0)	0.063	1.965 (1.039-3.716)
Chronic renal failure	8 (4.0)	3 (3.0)	5 (4.9)	0.506	
Chronic heart disease	33 (16.3)	13 (13.1)	20 (19.4)	0.227	
Chronic Obstructive Pulmonary Disease	19 (9.4)	5 (5.1)	9 (8.7)	0.302	
CCI, median (IQR)	6 (3.75-8.0)	6 (8-4)	6 (8-3)	0.048*	
Previous surgery, N (%)	90 (44.6)	43 (46.2)	47 (43.1)	0.657	
Internal stent, N (%)	47 (23.3)	26 (26.3)	21 (20.4)	0.323	
Percutaneous transhepatic catheterization (PTC), N (%)	139 (68.8)	72 (72.7)	67 (65)	0.239	
Site of acquisition, N (%)					
Community-acquired infection	82 (40.6)	33 (33.3)	49 (47.6)	0.039	1.515 (0.83-2.74)
Hospital-acquired infection	120 (59.4)	66 (66.7)	54 (52.4)		
Previous antibiotic use	161 (79.7)	85 (85.9)	76 (73.8)	0.033	2.268 (1.09-4.68)
Hospitalization in the last 3 months	112 (55.4)	62 (62.6)	50 (48.5)	0.044	1.628 (0.89-2.94)

Abbreviation: CCI; Charlson comorbidity index

* Variable with high correlation with other variables was not included in the regression model. CCI was not included in the model due to its high correlation with gastrointestinal malignancy, (Spearman, rho>0.75)

** Gastrointestinal malignancies include gastric cancer, hepatocellular carcinoma, biliary tract cancer, colorectal cancer, pancreatic cancer, and small intestine cancer.

Table 2. The outcomes of the study

	All of the patients N = 202	**MDR N = 99**	**Non-MDR N = 103**	**p-value**
Inappropriate empirical antibiotherapy*, N (%)	161	69 (56.6)	16 (41.0)	0.091
Duration of antibiotic treatment **, median (IQR)	11 (6.5-16.5)	13 (8-18)	8 (6-15)	0.054
Length of stay (days), median (IQR)	19 (12-31)	24 (15-32)	15 (9-30)	0.001
In-hospital mortality, N (%)	50 (24.8)	27 (27.3)	23 (22.3)	0.416

* It includes 161 patients who received empiric antibiotic therapy

** It includes 121 patients who received targeted antibiotic therapy

it was not statistically significant (13 days (8-18) vs. 8 days (6-15), p=0.054). In the MDR group, the median length of stay was longer than in the non-MDR group, and this difference was statistically significant (24 days (15-32) vs. 15 days (9-30), p=0.001). In the MDR group, in-hospital mortality was higher than in the non-MDR group, but it was not statistically significant (27.3% (n=27) vs. 22.3% (n=23), p=0.416).

A total of 342 microorganisms were isolated from 202 patients (Table 3). A single microorganism was isolated in 45.5% (n: 90), and two or more microorganisms in 55.5% (n: 112) of biliary samples. 64.3% (n: 220) of the isolates were Gram negatives, 27.5% (n: 94) were Gram positives, and 8.2% (n: 28) were fungi. The most common isolated Gram negative microorganisms were *E. coli* 37.2% (n=82), *Klebsiella* spp. 23.6% (n=52) and *Aeromonas* spp. 7.7% (n=17), respectively. The most common Gram positive microorganism was *Enterococcus* spp. 70.2% (n=66).

Table 3. Microorganisms isolated from biliary samples

Microorganisms, N	**N =342**
Gram Negative Organisms, N (%)	220 (64.3)
Escherichia coli, N (%)	82 (37.2)
MDR, N (%)	58 (70.0)
Klebsiella spp., N (%)	52 (23.6)
MDR, N (%)	26 (50.0)
Enterobacter spp, N (%)	15 (6.8)
MDR, N (%)	8 (53.3)
Citrobacter spp., N (%)	9 (4.1)
MDR, N (%)	2 (22.2)
Pseudomonas aeruginosa, N (%)	15 (6.8)
MDR, N (%)	5 (33.3)
Acinetobacter baumannii, N (%)	10 (45.4)
MDR, N (%)	7 (70.0)
Aeromonas spp, N (%)	17 (7.7)
MDR, N (%)	3 (17.6)
Stenotrophomonas maltophlia, N (%)	7 (3.2)
Others*, N (%)	13 (5.9)
Gram Positive Organisms, N (%)	94 (27.5)
Enterococcus spp., N (%)	66 (70.2)
MDR, N (%)	5 (7.6)
Streptococcus spp., N (%)	14 (14.9)
MDR, N (%)	0 (0)
Staphylococcus spp., N (%)	14 (14.9)
MDR, N (%)	2 (50)
Fungus, N (%)	28 (8.2)
Two or more microorganisms, N (%)	112 (55.5)
Single microorganism, N (%)	90 (44.5)
Antibiotic resistance	
Multidrug-resistant organisms, N / total (%)	119/281 (42.3)
ESBL (+) **, N / total (%)	93/169 (55.0)

* *Serratia* spp., *Proteus* spp., *Hafnia alvei*, *Morganella morganii*, *Burkholderia* spp., *Shewanella* spp.

** EBSL positivity was evaluated in *Enterobacterales* and determined by Ceftriaxone resistance.

DISCUSSION

The frequency of MDR microorganisms in biliary samples was significantly higher in patients with gastrointestinal malignancy and previously used antibiotics. MDR positivity was related to inappropriate empirical antibiotic treatment and lengthened duration of antibiotic use and hospitalization.

E. coli, *Enterococcus* spp., and *Klebsiella* spp. were the most frequently isolated microorganisms in our study. MDR positivity rate was frankly higher in *Klebsiella*, *Acinetobacter*, *Pseudomonas*, and *Enterobacter* species. These high rates of resistance among Gram negatives were compatible with national surveillance data on nosocomial infections (1). At least one MDR microorganism was detected in half of the patients. This rate was higher than in previously published studies due to the high prevalence of gastrointestinal malignancy and previous antibiotic use among the patient populations in our study (10, 11). Ozturk-Engin et al. reported 31% of MDR microorganism positivity in patients with cholecystectomy in Türkiye in 2019-2020 (10). It was 14.9% in the study of Reiter FP et al. However, the distribution of agents resistance patterns varied according to predisposing conditions (11). The frequency of Gram-negative microorganisms increased over the years (3). Serra Nikola et al. found Gram negative microorganism dominancy in biliary microbiome in elderly patients (2). In our study, the rate of multidrug-resistance among Gram negatives was higher in elderly patients with multiple comorbidities. Gastrointestinal malignancy increased approximately 2-fold the frequency of MDR positivity. Bogdan Miutescu et al. found an increased number of multidrug-resistance Gram negative (MDR-GN) strains in patients with malignancy (12). Furthermore, Amanati et al. reported that MDR-GN related infections increased over the years in patients with malignancy due to their repeated hospitalizations, chemotherapy, antibiotic use, and invasive procedures (13).

Another major risk factor for MDR microorganism-related infections is previous antibiotic use, especially within the last three months. This risk varies according to the used antibiotic classes (14-16). Male gender, nosocomial infection, previous antibiotic use, and presence of biliary stints were found as risk factors for MDR microorganisms in biliary samples, in a study of Reuken PA et al. (4). When we evaluate similar risk factors, antibiotic use within the last three months was found as an independent risk factor (2-fold) for the development of MDR microorganisms.

MDR microorganism positivity in preoperative biliary samples was a significant risk factor for mortality. It was associated with shorter survival in patients undergoing pancreatoduodenectomy for ductal adenocarcinoma (5, 6). Known colonization with MDR bacteria was a predictor for focal or systemic infection in patients with abdominal and gynecological malignancies (8, 17). MDR bacteria growth in biliary samples were related to subsequent bloodstream infection with the same microorganism (7, 18). Therefore, all patients with MDR bacteria in biliary samples, either colonized or infected, were included in our study. The duration of hospitalization was longer in patients with MDR bacteria. Inappropriate empirical antibiotic use, the total amount and duration of antibiotics, and the mortality rate were also higher in this group. However, statistical significance could not be demonstrated due to insufficient sample size.

Our study was underpowered for some outcomes, and this was one of the major limitations. The power was calculated as 60.3% for inappropriate empirical antibiotic use, 61.9% for the total amount of antibiotics, and 12.8% for mortality. Due to the single-centered and retrospective design of the study, the sample size was inadequate despite all colonized and infected patients were included.

In conclusion, gastrointestinal malignancy and antibiotic use within the last three months were significant risk factors for MDR positivity in biliary samples. This condition was associated with inappropriate antibiotic treatment. The duration of treatment and hospitalization increased in these patients. Therefore, screening and treatment algorithms for MDR-bacteria should be established.

CONCLUSION

MDR-bacteria positivity is associated with inappropriate antibiotic treatment, prolonged hospitalization, and increased mortality. Screening, antibiotic prophylaxis, and empirical treatment approaches should be carefully performed in patients with malignancy and previous antibiotic use in the last three months, which are significant risk factors for MDR infection or colonization.

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