Original Article

Developing a risk prediction model for multidrug-resistant bacterial infection in patients with biliary tract infection

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Abstract

Background/Aims: The aim of this study was to develop a tool to predict multidrug-resistant bacteria infections among patients with biliary tract infection for targeted therapy.

Patients and Methods: We conducted a single-center retrospective descriptive study from January 2016 to December 2018. Univariate and multivariable logistic regression analysis were used to identify independent risk factors of multidrug-resistant bacterial infections. A nomogram was constructed according to multivariable regression model. Moreover, the clinical usefulness of the nomogram was estimated by decision curve analysis.

Results: 121 inpatients were randomly divided into a training cohort ($n = 79$) and validation cohort ($n = 42$). In multivariate analysis, 5 factors were associated with biliary tract infections caused by multidrug-resistant bacterial infections: aspartate aminotransferase (Odds ratio (OR), 13.771; 95% confidence interval (CI), 3.747-64.958; $P < 0.001$), previous antibiotic use within 90 days (OR, 4.130; 95% CI, 1.192-16.471; $P = 0.032$), absolute neutrophil count (OR, 3.491; 95% CI, 1.066-12.851; $P = 0.046$), previous biliary surgery (OR, 3.303; 95% CI, 0.910-13.614; $P = 0.079$), and hemoglobin (OR, 0.146; 95% CI, 0.030-0.576; $P = 0.009$). The nomogram model was constructed based on these variables, and showed good calibration and discrimination in the training set [area under the curve (AUC), 0.86] and in the validation set (AUC, 0.799). The decision curve analysis demonstrated the clinical usefulness of our nomogram. Using the nomogram score, high risk and low risk patients with multidrug-resistant bacterial infection could be differentiated.

Conclusions: This simple bedside prediction tool to predict multidrug-resistant bacterial infection can help clinicians identify low versus high risk patients as well as choose appropriate, timely initial empirical antibiotics therapy. This model should be validated before it is widely applied in clinical settings.

Keywords: Biliary tract infection, multidrug-resistant bacterial, nomogram

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INTRODUCTION

Biliary surgical disease is a common disease in China, and most patients have complications of biliary tract infections (BTIs). Biliary tract infection is a bacterial infection in the biliary tract system, including acute or chronic cholecystitis and cholangitis.[1] Administration of appropriate antibiotics and biliary drainage are the mainstays of treatment for a BTI. Therefore, initial selection of appropriate antibiotics is of paramount importance in the process of treating a BTI.

Antimicrobial therapy largely depends on local antimicrobial susceptibility data. The extensive use of broad-spectrum antibiotics in previous years has promoted the rapid development of multidrug-resistant organisms (MDRO). In several studies performed throughout the world, the percentage of MDRO isolated from the bile of patients with acute cholangitis exceeded 20-30%.[2] The emergence of antimicrobial resistance has impacted the selection of empirical therapy for patients with intra-abdominal infections including acute cholangitis and cholecystitis.[3] The empiric antibiotic treatment strategies recommended by the existing guidelines were only successful in covering the resistant microorganisms in only a minority of cases. Moreover, biliary tract infection caused by antibiotic-resistant bacteria is usually complex and difficult to treat, and infection with antibiotic-resistant bacteria is an independent risk factor for organ failure and death in patients with biliary tract infections.[4] Therefore it is difficult for clinicians to choose an effective empiric antibacterial treatment strategy for antibiotic-resistant bacteria in a timely fashion in the treatment of biliary tract infection.

The use of severity of illness as a guide to antimicrobial agent selection has been questioned in the face of the increasing number of antibiotic-resistant bacteria in the community. These organisms are not reliably susceptible to the first-line antibiotic therapy. The criteria to determine the severity of biliary tract infection is based on whether the function of important organs of the body is damaged or not, and whether the hemodynamics are stable. However, the selection of antimicrobial agents should consider targeted organisms, pharmacokinetics and pharmacodynamics, local antibiogram, a history of antimicrobial usage, renal and hepatic function, and a history of allergies and other adverse events.[5] Towards this, it is necessary to understand the risk factors associated with BTI caused by MDRO, in order to develop the appropriate empiric antimicrobial treatment strategies. We believe that it would be useful for clinicians to use these risk factors as an objective bedside tool to start empiric broad-spectrum antibiotic in highly susceptible critically ill patients and avoid antibiotic over-exposure in those at low risk.

Although some previous studies[6,7] have identified independent risk factors associated with antibiotic-resistant bacteria in biliary tract infections, they were limited by focusing on specific bacteria, drugs or groups of patients. Further, without aggregating these risk factors, the utility of this information at the bedside is limited. Therefore, if a simple prediction tool would facilitate more accurate identification of MDRO in these patients, we could incorporate it within clinical care of patients to guide judicious empiric antibiotic therapy. A nomogram is used to transform statistical equations into simplified graphs and it has become a reliable and convenient tool for quantifying risk. However, using nomograms for predicting the risk of multidrug-resistant bacterial infections in biliary tract infection patients have been rarely reported.

In the current study, using clinical, demographic and therapeutic observations, we aim to develop a simple bedside prediction tool employing nomogram for predicting the risk of multidrug-resistant bacterial infections in the biliary tract in order to help clinicians with selection of empiric antibiotics for patients on admission.

PATIENTS AND METHODS

Study population

This retrospective, observational cohort study was conducted in Mengchao Hepatobiliary Hospital (tertiary specialist hospitals) of Fujian Medical University, Eastern China. Hospital records of patients treated from January 2016 through December 2018 were searched to identify all adult patients diagnosed with biliary tract infection. The inclusion criteria were as follows: (1) patients with community-acquired biliary tract infection (biliary tract infection diagnosed according to Tokyo Guidelines[8] and combined with clinical presentation, laboratory data and imaging findings); (2) bile specimens obtained at the beginning of surgery or endoscopic retrograde cholangiopancreatography (ERCP), or percutaneous transhepatic biliary drainage (PTBD). Patients who met any of the following criteria were excluded from analysis: (1) patients with hospital-acquired biliary tract infection; (2) bile specimens not obtained at the beginning of any procedure performed; (3) incomplete clinical data. Patients with polymicrobial infections were counted as a single infection for the analysis. If the patient was hospitalized and operated on multiple times during the study period, each hospitalization was analyzed as a separate case. Community-acquired infections were defined as biliary tract

infections that occurred with an onset before or within the first 48h of hospitalization. Hospital-acquired infection was defined as a biliary tract infection occurring 48h or longer after hospital admission.

Data collection

We retrospectively collected clinical and laboratory data on admission from the available medical records and included the following variables: age, gender, weight, comorbidities, length of hospital stay, cause of infection, previous hospitalization (within 90 days), previous antibiotic use (within 90 days), history of previous cholecystectomy, Charlson comorbidity index (CCI), fever (fever >38°C for more than two days), liver function tests, albumin, white blood cell count, neutrophil count, lymphocyte count, red blood cell count, hemoglobin, platelet count, mean platelet volume, C-reactive protein (CRP), procalcitonin (PCT), serum creatinine, prothrombin time (PT), international normalized ratio (INR), prothrombin activity (PTA), and ultrasonography (USG) or computed tomography findings. Antibiotics susceptibility tests were extracted from microbiologic laboratory database. Multidrug-resistant bacteria were defined as being non-susceptible to ≥1 agent in ≥3 antimicrobial categories. The antimicrobial categories were counted independently for each organism.[9]

Statistical analysis

The Kolmogorov-Smirnov test was performed to assess the distribution equality of continuous parameters. Normally distributed data are presented as the means ± standard deviations, while skewed distribution data are presented as median and interquartile range. Independent *t*-test and Mann-Whitney U test were used to analyze differences in continuous variables between groups in univariate analysis, while chi-square test and the Fisher's exact test were used for categorical variables. A receiver operating characteristic (ROC) curve analysis was performed to select the optimal cut-off value for the various continuous clinicolaboratory characteristics. Univariate and multivariate logistic regression analysis were performed to acquire the independent risk factors of the predictive model. The nomogram was constructed based on the result of multivariable analyses in the training cohort. The predictive performance of nomogram was measured through discrimination and calibration analysis. Discrimination of models was evaluated through the ROC curve[10]; calibration of models was measured by Hosmer-Lemeshow goodness-of-fit test and calibration plot. We used decision curve analysis, a method that incorporates clinical consequences, to evaluate the clinical utility of the multidrug-resistant bacterial infections prediction model. All statistical tests were performed using R statistical software version 3.5.3. Statistical significance was assumed at $P < 0.05$.

RESULTS

Patient characteristics

A total of 121 inpatients who met the enrollment criteria were included in the study and 45 (37.2%) were with polymicrobial infections. They were randomly divided into a training cohort ($n = 79$) and a validation cohort ($n = 42$) in a 60:40 ratio. To ensure the consistency of sampling results, we set seed = 43. (By setting the randomization seed, we ensured the repeatability of the results and facilitated others to use this data set for verification later.) Comparison of the clinicopathologic characteristics between training and validation cohorts is shown in Table 1. There were no significant differences in clinicopathologic features between the 2 cohorts.

Risk factors associated with multidrug-resistant bacterial infections

In the training cohort, 30 clinical basic indicators were included in the univariate analysis for multidrug-resistant bacterial infections, which showed that Charlson comorbidity index, procalcitonin, white blood cell count, absolute neutrophil count, lymphocyte count, hemoglobin, prothrombin time, alkaline phosphatase (ALP), and aspartate aminotransferase (AST) is associated with multidrug-resistant bacteria infections ($P \leq 0.1$) [Table 2]. At the same time, according to clinical experience and literature reports, the following two factors were also included in the multivariate analysis – previous antibiotic use within 90 days and previous biliary surgery.

By backward stepwise multivariable logistic regression analysis, the following factors were shown to be independently associated with multidrug-resistant bacterial infections: absolute neutrophil count, hemoglobin, AST, previous antibiotic use within 90 days, and previous biliary surgery [Table 3].

Construction of the multidrug-resistant bacterial infections-predicting nomogram

A nomogram was developed to predict multidrug-resistant bacterial infections in biliary tract infection patients based on the independently associated risk factors identified in the multivariate logistic model [Figure 1]. Each factor was assigned a weighted number of points. The total number of points for each patient was calculated using the nomogram and was associated with an estimated probability for multidrug-resistant bacterial infections. The AST level had the highest score (100 points). The scores for the other variables were: hemoglobin level (73.467

Table 1: Characteristics of patients in the training and validation cohorts

	Training Cohort (*n*=79)	Validation Cohort (*n*=42)	*P*
Age, years (mean, ±SD)	60.7 (12.9)	63.5 (13.8)	0.267
Gender, *n* (%)			0.299
Female	34 (43.0%)	23 (54.8%)	
Male	45 (57.0%)	19 (45.2%)	
Charlson comorbidity index, median (IQR)	3.00 (3.00)	3.00 (4.00)	0.751
Hypertension, *n* (%)			0.969
No	60 (75.9%)	31 (73.8%)	
Yes	19 (24.1%)	11 (26.2%)	
Diabetes, *n* (%)			0.946
No	64 (81.0%)	35 (83.3%)	
Yes	15 (19.0%)	7 (16.7%)	
Cirrhosis, *n* (%)			0.82
No	65 (82.3%)	36 (85.7%)	
Yes	14 (17.7%)	6 (14.3%)	
Hypoproteinemia, *n* (%)			1
No	69 (87.3%)	36 (85.7%)	
Yes	10 (12.7%)	6 (14.3%)	
Previous antibiotic use within 90 days			0.333
No	48 (60.8%)	30 (71.4%)	
Yes	31 (39.2%)	12 (28.6%)	
Previous biliary surgery			1
No	51 (64.6%)	27 (64.3%)	
Yes	28 (35.4%)	15 (35.7%)	
Fever			0.776
No	53 (67.1%)	30 (71.4%)	
Yes	26 (32.9%)	12 (28.6%)	
Abdominal pain			0.962
No	30 (38.0%)	15 (35.7%)	
Yes	49 (62.0%)	27 (64.3%)	
Murphy's sign			0.605
No	63 (79.7%)	31 (73.8%)	
Yes	16 (20.3%)	11 (26.2%)	
Bile specimen sources			0.798
ERCP	15 (19.0%)	6 (14.3%)	
PTCD	17 (21.5%)	9 (21.4%)	
Surgery	47 (59.5%)	27 (64.3%)	
Causes of biliary obstruction, *n* (%)			0.317
Gallstone	69 (87.3%)	33 (78.6%)	
Tumor	10 (12.7%)	9 (21.4%)	
C-reactive protein (mg/L)			0.165
≤76.81	53 (67.1%)	22 (52.4%)	
>76.81	26 (32.9%)	20 (47.6%)	
Procalcitonin (ng/ml)			0.523
≤0.35	42 (53.2%)	19 (45.2%)	
>0.35	37 (46.8%)	23 (54.8%)	
White blood cell count ($\times 10^9$/L)			0.083
≤10.125	62 (78.5%)	26 (61.9%)	
>10.125	17 (21.5%)	16 (38.1%)	
Absolute neutrophil count ($\times 10^9$/L)			0.402
≤4.57	32 (40.5%)	13 (31.0%)	
>4.57	47 (59.5%)	29 (69.0%)	
Lymphocyte count ($\times 10^9$/L)			0.846
≤1.56	61 (77.2%)	31 (73.8%)	
>1.56	18 (22.8%)	11 (26.2%)	
Red blood cell count ($\times 10^{12}$/L)			1
≤3.665	14 (17.7%)	8 (19.0%)	
>3.665	65 (82.3%)	34 (81.0%)	
Hemoglobin (g/L)			0.322
≤114.5	20 (25.3%)	15 (35.7%)	
>114.5	59 (74.7%)	27 (64.3%)	
Platelet count ($\times 10^9$/L)			0.546
≤170	28 (35.4%)	18 (42.9%)	
>170	51 (64.6%)	24 (57.1%)	
Mean platelet voulume (fL)			0.356
≤9.65	46 (58.2%)	20 (47.6%)	

Contd...

Table 1: Contd...

	Training Cohort (*n*=79)	Validation Cohort (*n*=42)	*P*
>9.65	33 (41.8%)	22 (52.4%)	
Prothrombin time (second)			0.204
≤13.25	43 (54.4%)	17 (40.5%)	
>13.25	36 (45.6%)	25 (59.5%)	
Albumin (g/L)			0.785
≤39.5	55 (69.6%)	31 (73.8%)	
>39.5	24 (30.4%)	11 (26.2%)	
Total bilirubin (μmol/L)			0.862
≤85.2	46 (58.2%)	23 (54.8%)	
>85.2	33 (41.8%)	19 (45.2%)	
Alkaline phosphatase (U/L)			0.821
≤230.5	37 (46.8%)	18 (42.9%)	
>230.5	42 (53.2%)	24 (57.1%)	
Gamma-glutamyl transferase (U/L)			0.843
≤275.5	33 (41.8%)	16 (38.1%)	
>275.5	46 (58.2%)	26 (61.9%)	
Alanine aminotransferase (U/L)			0.365
≤83.5	44 (55.7%)	19 (45.2%)	
>83.5	35 (44.3%)	23 (54.8%)	
Aspartate aminotransferase U/L)			0.848
≤82	48 (60.8%)	24 (57.1%)	
>82	31 (39.2%)	18 (42.9%)	

Table 2: Risk factors for multidrug-resistant bacterial infections in the training cohort

	Without multidrug-resistant bacteria (*n*=41)	With multidrug-resistant bacteria (*n*=38)	*P*
Age, years (mean, ±SD)	59.9 (12.9)	61.5 (13.0)	0.598
Gender, *n* (%)			0.698
Female	19 (46.3%)	15 (39.5%)	
Male	22 (53.7%)	23 (60.5%)	
Charlson comorbidity index, median (IQR)	3.00 (3.00)	4.00 (2.00)	**0.092**
Hypertension, *n* (%)			0.849
No	32 (78.0%)	28 (73.7%)	
Yes	9 (22.0%)	10 (26.3%)	
Diabetes, *n* (%)			0.87
No	34 (82.9%)	30 (78.9%)	
Yes	7 (17.1%)	8 (21.1%)	
Cirrhosis, *n* (%)			0.103
No	37 (90.2%)	28 (73.7%)	
Yes	4 (9.8%)	10 (26.3%)	
Hypoproteinemia, *n* (%)			0.64
No	37 (90.2%)	32 (84.2%)	
Yes	4 (9.8%)	6 (15.8%)	
Previous antibiotic use within 90 days			0.233
No	28 (68.3%)	20 (52.6%)	
Yes	13 (31.7%)	18 (47.4%)	
Previous biliary surgery			0.339
No	29 (70.7%)	22 (57.9%)	
Yes	12 (29.3%)	16 (42.1%)	
Fever			0.339
No	30 (73.2%)	23 (60.5%)	
Yes	11 (26.8%)	15 (39.5%)	
Abdominal pain			1
No	16 (39.0%)	14 (36.8%)	
Yes	25 (61.0%)	24 (63.2%)	
Murphy's sign			0.912
No	32 (78.0%)	31 (81.6%)	
Yes	9 (22.0%)	7 (18.4%)	
Bile specimen sources			0.103
ERCP	6 (14.6%)	9 (23.7%)	
PTCD	6 (14.6%)	11 (28.9%)	
Surgery	29 (70.7%)	18 (47.4%)	
Causes of biliary obstruction, *n* (%)			0.252
Gallstone	38 (92.7%)	31 (81.6%)	
Tumor	3 (7.3%)	7 (18.4%)	

Contd...

Table 2: Contd...

	Without multidrug-resistant bacteria (*n*=41)	With multidrug-resistant bacteria (*n*=38)	*P*
C-reactive protein (mg/L)			0.151
≤76.81	31 (75.6%)	22 (57.9%)	
>76.81	10 (24.4%)	16 (42.1%)	
Procalcitonin (ng/ml)			**0.095**
≤0.35	26 (63.4%)	16 (42.1%)	
>0.35	15 (36.6%)	22 (57.9%)	
White blood cell count (×10^9/L)			**0.069**
≤10	36 (87.8%)	26 (68.4%)	
>10	5 (12.2%)	12 (31.6%)	
Absolute neutrophil count (×10^9/L)			**0.074**
≤4.57	21 (51.2%)	11 (28.9%)	
>4.57	20 (48.8%)	27 (71.1%)	
Lymphocyte count (×10^9/L)			**0.026**
≤1.56	27 (65.9%)	34 (89.5%)	
>1.56	14 (34.1%)	4 (10.5%)	
Red blood cell count (×10^{12}/L)			0.103
≤3.665	4 (9.8%)	10 (26.3%)	
>3.665	37 (90.2%)	28 (73.7%)	
Hemoglobin (g/L)			**0.002**
≤114.5	4 (9.8%)	16 (42.1%)	
>114.5	37 (90.2%)	22 (57.9%)	
Platelet count (×10^9/L)			0.627
≤170	13 (31.7%)	15 (39.5%)	
>170	28 (68.3%)	23 (60.5%)	
Mean platelet voulume (fL)			0.531
≤9.65	22 (53.7%)	24 (63.2%)	
>9.65	19 (46.3%)	14 (36.8%)	
Prothrombin time (second)			**0.001**
≤13.25	30 (73.2%)	13 (34.2%)	
>13.25	11 (26.8%)	25 (65.8%)	
Albumin (g/L)			0.136
≤39.5	25 (61.0%)	30 (78.9%)	
>39.5	16 (39.0%)	8 (21.1%)	
Total bilirubin (μmol/L)			0.138
≤85.2	28 (68.3%)	18 (47.4%)	
>85.2	13 (31.7%)	20 (52.6%)	
Alkaline phosphatase (U/L)			**0.017**
≤230.5	25 (61.0%)	12 (31.6%)	
>230.5	16 (39.0%)	26 (68.4%)	
Gamma-glutamyl transferase (U/L)			0.279
≤275.5	20 (48.8%)	13 (34.2%)	
>275.5	21 (51.2%)	25 (65.8%)	
Alanine aminotransferase (U/L)			0.227
≤83.5	26 (63.4%)	18 (47.4%)	
>83.5	15 (36.6%)	20 (52.6%)	
Aspartate aminotransferase (U/L)			**0.002**
≤82	32 (78.0%)	16 (42.1%)	
>82	9 (22.0%)	22 (57.9%)	

points), previous antibiotic use within 90 days (54 points), absolute neutrophil count level (47.66 points), and previous biliary surgery (45.56 points).

Assessment of model performance

The evaluation of the regression model performance is shown in Figure 2. The variance inflation factor (VIF) values of the 5 covariates ranged from 1.08 to 1.53, indicating no collinearity in the model. Figure 2c shows a calibration curve for the regression model in the training cohort. The calibration curve and Hosmer-Lemeshow test (P = 0.896) performed well in the training cohort. An area under the curve (AUC) of 0.860 (95% CI, 0.779-0.941) indicated good differentiation ability of the model in the training cohort [Figure 2a]. The calibration map of the multidrug-resistant bacterial infections regression model was confirmed using the validation cohort [Figure 2d]. The Hosmer-Lemeshow test showed that the P value was not significant at 0.724, and the AUC of the validation group [Figure 2b] was 0.799 (95% CI, 0.660-0.938), suggesting good performance of the model in both the training and validation cohorts.

Clinical implementation of the prediction model: Net benefit analysis

The prediction of a model with an AUC of 0.86 is good, but not perfect. Therefore, a net benefit analysis

Table 3: Multivariate logistic analyses of risk factors for multidrug-resistant bacterial infections in the training cohort

Clinical Variables	β	OR (95%CI)	*P*
AST, ≤82 vs >82 U/L	2.623	13.771 (3.747-64.958)	<0.001
HB, ≤114.5 vs >114.5 g/L	-1.927	0.146 (0.030-0.576)	0.009
ANC, ≤4.57 vs >4.57×10^9/L	1.250	3.491 (1.066-12.851)	0.046
Previous antibiotic use within 90 days, yes vs no	1.418	4.130 (1.192-16.471)	0.032
Previous biliary surgery, yes vs no	1.195	3.303 (0.910-13.614)	0.079

AST: Aspartate aminotransferase; HB: Hemoglobin; ANC: Absolute neutrophil count; β: Unstandardized βcoefficients were calculated from the multivariate logistic regression model

was performed to evaluate under which conditions use of the model provides higher benefit than alternative strategies. A decision curve shows the relative net benefit over a range of threshold probabilities from 0 to 0.85 [Figure 3] for 3 strategies: (i) carbapenems, piperacillin/tazobactam, tigecycline and aminoglycosides were selected as empirical antimicrobial therapy for patients with biliary tract infection (treat all); (ii) carbapenems, piperacillin/tazobactam, tigecycline and aminoglycosides were not selected as empirical antimicrobial therapy for patients (treat none); (iii) prescribe empirical antimicrobial therapy by carbapenems, piperacillin/tazobactam, vancomycin and aminoglycosides only for patients with a predicted risk of multidrug-resistant bacterial infections exceeding the threshold. It can be observed that the net benefit of treating according to the model is all higher than the net benefit of the alternative strategies for thresholds between 0.2 and 0.85 in both cohorts.

Clinical utility of nomogram for multidrug-resistant bacterial infections

To allow the nomogram more plausible and easier to utilize in clinical practice, we looked at different cut-off values with interpretability according to our knowledge and practice. The total nomogram scores of each patient were calculated based on the nomogram, and ROC curve analysis was used to calculate the sensitivity and specificity of different cut-off values. The optimal cut-off values for predicting multidrug-resistant bacterial infections were determined according to a positive likelihood ratio (PLR) of nearly 10.0 for high risk group and a negative likelihood ratio (NLR) of nearly 0.1 for low risk group.[11]

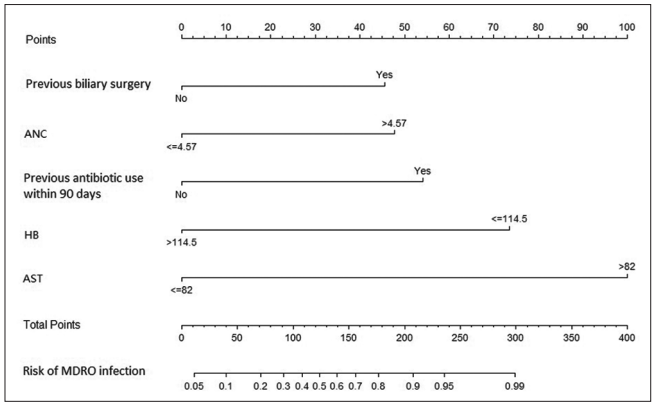

Figure 1: Nomogram predicting the probability of multidrug-resistant bacterial infections in biliary tract infection patients

For our study, the high risk cut-off value of 160.34 showed a PLR of 9.17, a specificity of 95.1% and the low risk cut-off value of 73.61 showed a NLR of 0.11, and a sensitivity of 94.7%. By using these two cut-off values 73.61 and 160.34, we identified 3 groups: low risk group (≤73.61), medium risk group (73.61 < nomogram score < 160.34), and high risk group (≥160.34).

Table 4 shows that the positive predictive values of the high risk group of the multidrug-resistant bacteria infection prediction model in the training cohort and the validation cohort were 89.5% and 86.7%, respectively. The negative predictive value of low risk group was 90.5% in the training cohort and 73.3% in the validation cohort. The prediction model can well distinguish high risk group and low risk group, and provide basis for the selection of empiric antimicrobial agents.

DISCUSSION

The over-usage of antibiotics increases the risk of infection or colonization with multidrug-resistant bacteria, and at the same time inadequate definitive antimicrobial therapy is consistently associated with increased mortality in critically ill patients mainly due to the presence of resistant organisms. There is therefore a critical need to identify, at the bedside, which patients are at high risk for infection with multidrug-resistant pathogens, so that initial empiric therapy can be targeted at these patients without adversely affecting the rest of the patients with BTI. Unfortunately, many previous studies[6,7,12,13] have only identified a few risk factors that lead to multidrug-resistant bacterial infections in BTI patients, but no model has been constructed to predict the probability of multidrug-resistant bacterial infections in individual patients. We used a nomogram to build a simple, convenient and repeatable bedside prediction tool which predicts multidrug-resistant bacteria infection and differentiates low versus high risk patients. This prediction tool is easy to apply and can help clinicians

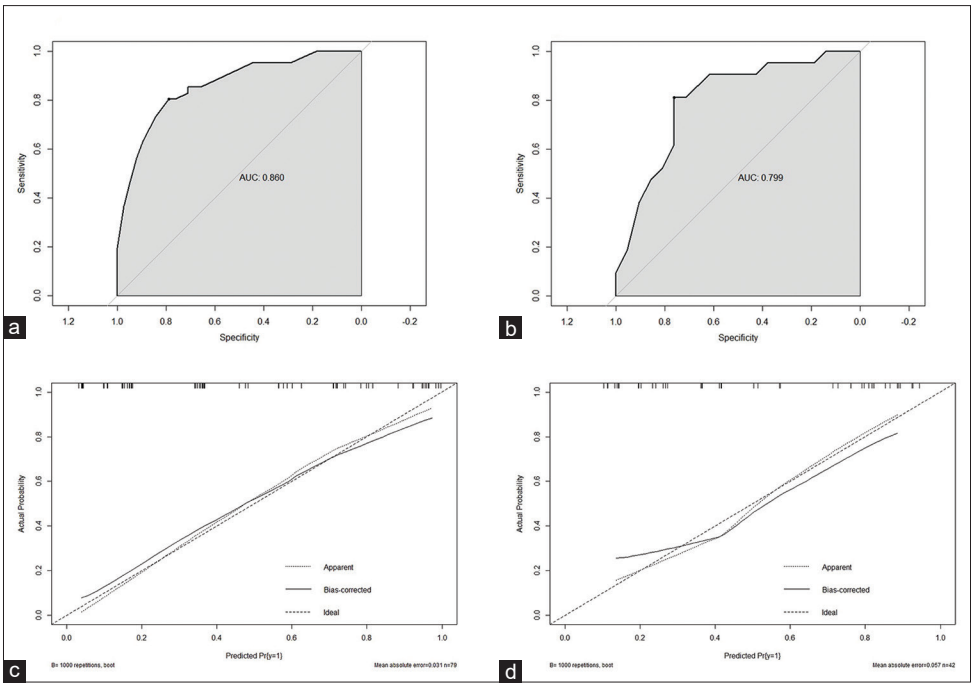

Figure 2: Goodness of fit of the predicted risk and actual risk of multidrug-resistant bacterial infections. (a) The ROC curves of the model in training sets. (b) the ROC curves of the model in validation sets. (c) Calibration curves of the model in the training set. (d) Calibration curves of the model in the validation set. ROC curves depict discrimination capability of nomogram model. The larger the area of the AUC, the higher the prediction accuracy of the model. Calibration curves depict the calibration of the model in terms of agreement between the predicted risk of multidrug-resistant bacterial infections and observed multidrug-resistant bacterial infections outcomes. The 45-degree long dotted line represents a perfect prediction, and the solid line represent the predictive performance of the model. The closer the long dotted line fit is to the ideal line, the better the predictive accuracy of the model

identify the patients at risk of multidrug-resistant bacterial infections who need early broad-spectrum appropriate antibiotics while reducing the risk of unnecessary antibiotic exposure. Striking this balance is crucial to reduce the emergence of antimicrobial resistance while averting mortality from infections. Moreover, since application of clinical evidence varies tremendously among clinicians,[14] the use of a bedside prediction tool would help narrow the variability between clinicians in decision-making especially in areas with high prevalence of drug-resistant bacteria.

The overall performance of the model is good, with AUC of 0.860 and 0.799 in the training set and the validation set, respectively. The calibration curve also showed that the predicted value was consistent with the actual value. Decision curve analysis showed that the net benefit of treating according to the model is higher than the net benefit of the alternative strategies within threshold probabilities between 0.2 and 0.85 in both cohorts. A prediction model gives a predicted probability directly, and doctors choose the appropriate threshold probability

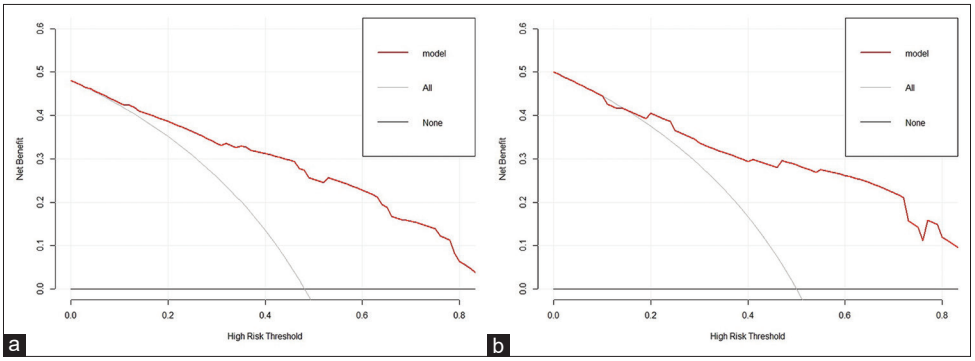

Figure 3: Decision curve analysis for predicting multidrug-resistant bacterial infections. The x-axis depicts the risk threshold probability that changes from 0 to 0.85, and the y-axis shows the calculated net benefit for a given threshold probability. The red curves depict the net benefit of the model. The gray lines display the net benefits in the alternative strategies of all patients with multidrug-resistant bacterial infections, and the black lines display the net benefits in the alternative strategies of no patients with multidrug-resistant bacterial infections. (a) Training cohort; (b) Validation cohort

Table 4: Positive and negative predictive values of different risk groups of multidrug-resistant bacterial infections predicted by a nomogram in a training cohort and a validation cohort

Risk Category	Training Cohort		Validation Cohort	
	Positive predictive value, %	Negative predictive value, %	Positive predictive value, %	Negative predictive value, %
Low	9.5	90.5	26.7	73.3
Medium	48.7	51.3	33.3	66.7
High	89.5	10.5	86.7	13.3

according to their own clinical experience. The chosen threshold probability serves as a cut-off value of predicted probability at which one decides whether to use empiric broad-spectrum antibacterial treatment. In order to narrow the decision-making differences between clinicians in our study, the optimal cut-off value was determined according to positive likelihood ratio (PLR) of nearly 10.0 for high risk group and negative likelihood ratio (NLR) of nearly 0.1 for low risk group.[11] The positive predictive value of the high risk group was 89.5%, while the negative predictive value of the low risk group was 90.5%, which indicated that the multidrug-resistant bacterial infections prediction model could distinguish the high risk and low risk patients. For high risk patients with multidrug-resistant bacterial infections, carbapenems, piperacillin/tazobactam, tigecycline and aminoglycosides are empirically recommended. For low risk patients, these drugs are not recommended to avoid exposure to unnecessary treatment, because some research has confirmed that even a short duration of carbapenems use in critically patients with illness increases the risk of infection or colonization with multidrug-resistant bacteria.[15] Reducing indiscriminate antibiotic usage in low risk patients can effectively slow down the emergence of multidrug-resistant bacteria.

In our setting, absolute neutrophil count, hemoglobin, AST, the previous antibiotic use within 90 days and previous biliary surgery were associated with a higher risk of contracting multidrug-resistant bacterial infections in the BTI patients. These five predictors are easy to obtain in clinical practice, even in developing countries, indicating that the model has a wide range of application. In the present study, the previous antibiotic use within 90 days and previous biliary surgery were not statistically significant in univariate analysis, but based on clinical experience and prior reports[5-7,12,13] we included these in the multivariate analysis. The results of multivariate analysis showed that previous antibiotic use within 90 days and previous biliary surgery were independent risk factors for multidrug-resistant bacterial infections. One possible reason for this increase in antibiotic resistance is that antibiotic exposure has increased due to abuse, misuse, or even appropriate use. After exposure to an antibiotic, a resistant subset of organisms may survive, proliferate, and become predominant. A study in Korea confirmed that the risk factors for biliary tract infection caused by extended-spectrum beta-lactamase-producing organisms were related to the history of antibiotic use within the previous 90 days, and that the patients at high risk were those who were previously prescribed carbapenems.[13] Some studies[12,16] have suggested that patients with a history of a biliary surgery were at increased risk of bacteriobilia, which may be due to these interventions, which change the anatomy of the bile duct, destroy the normal human defense mechanism, decrease the pressure of the bile duct, and may lead to bile reflux. With the reflux of bile increasing, biliary mucosa could experience inflammatory edema, ultimately generating a favorable environment for bacterial proliferation and colonization. Colonization with bacteria is a strong risk factor for subsequent infection with the organisms. Individuals with these interventions may be prone to biliary tract infection, increasing the exposure of antimicrobial agents, thereby increasing the production of resistant bacteria. In our study, 43 patients had a history of a biliary procedure, 14 of whom had cholecystectomy, and the other 29 patients had choledochotomy or partial hepatectomy in addition to cholecystectomy. The proportion of multidrug-resistant bacteria in the two groups was 57% and 62.1%, respectively. The difference between the 2 groups was not statistically significant. This further proves that the previous history of biliary tract surgery was a risk factor for multidrug-resistant bacterial infections which concurred with those of previous studies.[7,13]

In our study, an interesting phenomenon was found that low hemoglobin (≤114.5 g/L) was an independent risk factor for multidrug-resistant bacterial infections in patients with biliary tract infection. Others have also found that low hemoglobin level was significantly associated with the emergence of vancomycin-resistant enterococci[17] and was one of the risk factors for carbapenems resistant *Escherichia coli* infection.[18] The susceptible factors of multidrug-resistant bacterial infections include on the one hand, bacteria gaining stronger virulence through evolution thereby enhancing their ability to invade patients. Hemoglobin provides the most abundant source of iron in the human body and pathogens depend on acquiring

iron from hemoglobin to cause invasive disease. In order to persist, drug-resistant bacteria may be evolving to acquire iron acquisition systems to overcome the elaborate processes mammals use to withhold iron from pathogens during infection, which was observed in some other common bacterial pathogens.[19,20] On the other hand, the patient's general status determines whether they are susceptible to bacterial infection. Studies have indicated that low hemoglobin was one of the risk factors for biliary infection in patients after percutaneous transhepatic biliary drainage,[21] and increased risk of postoperative infection in patients with preoperative low hemoglobin.[22,23] It needs further research to confirm whether the patients with low hemoglobin are easy to get repeated infections, which increases the exposure of antibiotics and eventually leads to the emergence of drug-resistant bacteria.

Cholestasis is a key clinical feature of biliary tract infection. Jaundice is only observed in 60-70% of patients.[24] In the absence of jaundice, acute biliary tract infection can be diagnosed according to ALP, Gamma-glutamyl transferase (GTP), AST and alanine aminotransferase (ALT) in blood test results. Univariate analysis showed that the ALP and AST of patients with multidrug-resistant bacterial infections were significantly higher than those without multidrug-resistant bacterial infections. The multivariable logistic regression analysis showed that AST was the most influential variable (odds ratio = 13.77) in the model. The risk of multidrug-resistant bacterial infections in patients with AST >82 U/L was 13.77 times higher than that in patients with low AST level. In addition, systemic inflammation is one of the diagnostic criteria for acute biliary tract infection. We found that there were differences in white blood cell count and procalcitonin levels between the two groups. An absolute neutrophil count (ANC) greater than 4.57×10^9/L was an independent risk factor for multidrug-resistant bacterial infection.

Our study has some limitations. Firstly, as a retrospective study, a certain degree of selection bias may exist. We obtained data in a limited number of case observations from a small sample size and from a single center. Secondly, external validation of the model is required before it can be widely applied in the clinical setting. Prospective studies are also needed to verify the accuracy of our nomogram, and to try and find a more reasonable cut-off value to distinguish the high and low-risk patients in order to avoid unnecessary antimicrobial exposure. Thirdly, we were unable to obtain the past prescribed antibiotic classes of those patients with previous antibiotic use within 90 days. Therefore, we could not study whether the category of antibiotics is a risk factor of multidrug-resistant bacterial infection.

Our results demonstrated that 5 independent risk factors were associated with multidrug-resistant bacterial infections in the biliary tract infection patients. These 5 predictors were not only easy to obtain in clinical experiment, but can also explain the clinical significance of this model. This simple bedside prediction tool to predict multidrug-resistant bacterial infection can help clinicians identify low versus high risk patients as well as choose appropriate, timely initial empirical antibiotics therapy.

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Conflicts of interest

There are no conflicts of interest.

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