Development and validation of a nomogram for predicting carbapenem-resistant organism infection and prognostic risk factors analysis in elderly hospitalized patients

Yao Wang

First Affiliated Hospital of Jiamusi University

Huiling Li

First Affiliated Hospital of Jiamusi University

Ying Chen

First Affiliated Hospital of Jiamusi University

YanJun Fu

First Affiliated Hospital of Jiamusi University

Jianfeng Xi

First Affiliated Hospital of Jiamusi University

Yong Wang (✉ jmsuwy@163.com)

First Affiliated Hospital of Jiamusi University <https://orcid.org/0000-0002-0358-2259>

Research

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1 Development and validation of a nomogram for predicting 2 carbapenem-resistant organism infection and prognostic 3 risk factors analysis in elderly hospitalized patients

4 Yao Wang¹, Huiling Li¹, Ying Chen², Yanjun Fu¹, Jianfeng Xi¹, Yong Wang¹

5 ¹Department of Clinical Laboratory, The First Affiliated Hospital of Jiamusi University, Jiamusi, 154007, China. ²Department of Intensive
6 Care Unit, The First Affiliated Hospital of Jiamusi University, Jiamusi, 154007, China.

7 Correspondence:

8 Wang Yong, Department of Clinical Laboratory, The First Affiliated Hospital of Jiamusi University, Changan Road, Xiangyang District,
9 Jiamusi, 154007, China. E-mail: jmsuwy@163.com

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11 Abstract

12 **Background:** With the extensive use of carbapenem-resistant organism (CRO), CRO infection is
13 constantly detected clinically which has limited available drugs. There have many mechanisms of
14 CRO resistance and rapid horizontal transmission, while the elderly with low resistance is more
15 likely to acquire nosocomial infection. We aimed to screen elderly patients for nosocomial CRO
16 infection and potential risk factors for prognosis.

17 **Methods:** A total of 177 patients with CRO and carbapenem-sensitive organism (CSO) infection
18 were included in this study. A least absolute shrinkage and selection operator (LASSO) analysis
19 was used to select variables. The nomogram was constructed with multivariable logistic regression.
20 The performance of the model was assessed by the receiver operating characteristic(ROC) curve,
21 calibration, decision curve , and clinical influence curve. Using X-tile to stratify the prognosis risk,
22 Kaplan-Meier curve for risk assessment.

Results: Respiratory diseases, mechanical ventilation, indwelling urinary catheters , and APACHE II over 20 were selected as predictors of the CRO infection model. The model showed good discrimination and consistency in the training set and the validation set. The area under the ROC was 0.840 (95% CI: 0.773-0.900)in the training set and 0.822 (95% CI: 0.678-0.936) in the validation set. Decision analysis and influence curve showed that the model was clinically useful. Hepatobiliary diseases, indwelling urinary catheters, and hospital stays longer than 20 days were used as prognostic predictors. After analysis, the prognostic model demonstrated good discrimination of 0.817 (95% CI: 0.729-0.893) and consistency. Risk stratification showed the high-risk group had a poorer prognosis.

Conclusion: Predicting clinically relevant risk factors for CRO nosocomial infection and prognosis in elderly patients. This may help the treatment of clinical drug-resistant infections.

Keywords: CRO, nosocomial infection, nomogram, risk stratification, risk factors

Background

Normally, there are a large number of colonization bacteria in the human body and the hospital environment. Once the body is in a steady-state imbalance, it may cause conditional disease. Carbapenems are not only the first choice for the production of extended-spectrum β -lactamase (ESBLs) or cephalosporinase bacteria, but also the last line of defense for the treatment of clinical Gram-negative bacilli infections which have broad antibacterial spectrum and strong antibacterial activity. Due to the widespread and unreasonable use of carbapenem, the choice of clinical antibiotics is greatly limited, resulting in a more serious CRO nosocomial infection worldwide[1]. WHO and America Centers for Disease Control determine CRO's risk level as the primary[2],

Alessandro et al. [3] indicate that about 33,000 people die each year due to different bacterial infections, and 75% of the disease burden is attributed to infectious diseases. The status of CRO infection is not optimistic in China, the bacterial resistance monitoring report shows that the detection rate of CRO has been on the rise, the detection rate of Carbapenem-resistant *Klebsiella pneumoniae*(CRKP) does not exceed 4% in 2005, and have exceeded 25% by 2018[4].

CRO mainly includes Carbapenem-resistant *Enterobacteriaceae*(CRE), Carbapenem-resistant *Acinetobacter baumannii*(CRAB), and Carbapenem-resistant *Pseudomonas aeruginosa*(CRPA) which can cause a variety of infections with a high mortality rate. A meta-analysis shows that the mortality rate of CRO infection is twice that of sensitive bacteria[5, 6]. According to reports, the mechanism of resistance is mainly carbapenemase production, and the resistance gene can be rapidly transmitted between strains through mobile elements such as transposons, integrants, and plasmids[7]. Besides, CRO has strong survivability, and it is not possible to completely avoid infection under the premise of standardized operation[8].

As the population ages, the proportion of the elderly population gradually increases. On the one hand, the elderly have declining physiological function, reduced resistance and reaction ability to varying degrees, resulting in atypical clinical symptoms, rapid changes in disease status, long course of the disease, and the slow recovery of prognosis; not only that, there are many adverse drug reactions in the elderly, which are more likely to cause respiratory tract, urinary tract, blood flow and biliary tract infections[9, 10]; On the other hand, some elderly patients lack correct understanding of the disease, which can easily aggravate the mental burden and cause mental trauma and pain, which is not conducive to the prognosis of the disease.

Therefore, to understand the potential risk factors of elderly CRO infection and prognosis in the

eastern region of Heilongjiang Province, and to better assist in the prevention and control of clinical treatment, this study collected clinical data of elderly patients in a hospital in Heilongjiang Province, and screened the potential risk factors of CRO hospital infection and prognosis.

Methods

Data sources

CRO and CSO infected elderly patients were collected retrospectively in local hospitals in 2018. This hospital has more than 1,600 beds and a treatment center. Infection standards complied with "Hospital Infection Diagnostic Standards (Trial)"[\[11\]](#). Exclusion criteria: (1) Only the first CRO infection was included in the same patient; (2) CRO infection was detected within 48 hours after admission; (3) Discharge or death within 48 hours after admission; (4) Incomplete basic data.

Strain identification

Specimens were inoculated with OXOID blood plates and McConkey plates. French bioMérieux company VITEK-2 and Siemens Walk Away40Plus identified strains and drug susceptibility.

CRO infection analysis

The elderly patients with nosocomial infection were randomly divided into a training set and a validation set according to 8:2. The training set was used for risk factor screening and prediction model construction, and the validation set was used for model validation.

Prognostic analysis

According to the prognosis, CRO-infected patients were divided into a prognosis-improving group and poor-prognosis group. By screening the risk factors of poor prognosis, the risk values were stratified.

Statistical analysis

For statistics of bacteria, WHONET5.6 was used for analysis, and SPSS17.0 was used for general analysis. Risk factors were screened by R3.6.3 and a model was constructed. The measurement data was expressed as "mean $\pm$ standard deviation" and the normal test was performed. If the normal distribution was satisfied, the student's t-test was used, otherwise, the Mann-Whitney U test was performed. Frequency or percentage was used to represent the count data, statistical analysis was performed by the chi-squared test, $P < 0.05$ was statistically significant.

Variables were analyzed by Lasso, and multivariate logistic regression was used to screen clinical predictors. The model repeats self-sampling through 1000 times of Bootstrap, the distinguish ability was verified by ROC, and the calibration curve verified the degree of calibration. To calculate the net benefit at different threshold probabilities, the clinical decision curve and clinical impact curve were performed to evaluate the clinical utility of the CRO infection model. In addition, in order to further analyze the prognostic model, the risk score of the prognostic model was calculated to re-value the data, using X-tile software risk stratification, Kaplan-Meier curve risk assessment.

Results

Strain distribution

A total of CRO 93 strains were collected, mostly from ICU departments and sputum specimens, mainly *Acinetobacter baumannii* (66.7%), *Pseudomonas aeruginosa* (15.1%) and *Klebsiella pneumoniae* (7.5%)(Table 1).

Table 1 Strain distribution

Strain	Number(n)	Percentage(%)
<i>Acinetobacter baumannii</i>	62	66.7

Pseudomonas aeruginosa	14	15.1
Klebsiella pneumoniae	7	7.5
Other strains	10	10.8

Clinical features and characteristics

According to the inclusion criteria of this study, there were 93 cases in the CRO group and 84 cases in the CSO group. There was no significant difference in the admission history and length of hospitalization between the two groups. However, there were still significant differences in various aspects of data. The two groups had significant differences in the types and duration of drug use. The CRO group usually had decreased immune function and was accompanied by multiple underlying diseases. In addition, the CRO group has more ICU stays and long stays, and most of them accept invasive operations([Table 2, visible at the end of the article](#)).

CRO infection analysis

Developing a prediction model

To avoid over-fitting the model caused by high-dimensional data, we adjusted the penalty parameter lambda through Lasso and selected the optimal lambda through 10-fold cross-validation to obtain a non-zero coefficient variable([Fig. 1](#)). Finally, four risk factors were screened out through multi-factor Logistic regression: respiratory disease, mechanical ventilation, indwelling urinary catheter and APACHEII score > 20. The VIF between the above variables was less than 5 excluded multicollinearity([Table 3](#)). A predictive nomogram was developed to visualize the results([Fig. 2](#)).

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Table 3 Multivariate Logistic analysis

Risk factors	β	OR	95%CI	P-value
Respiratory diseases	1.221	3.389	1.003~11.475	0.0495
Mechanical Ventilation	1.383	3.988	1.645~9.668	0.0022
Indwelling catheter	0.950	2.587	1.022~6.549	0.0450
APACHEII score over 20	1.522	4.582	1.386~15.154	0.0126

Abbreviations: OR Odds Ratio, CI Confidence interval, APACHEII Acute Physiology and Chronic Health Evaluation II

126

127 **Validation of the nomogram**

Validation of the nomogram was repeated by 1000 bootstrap self-sampling. Both the training set and the validation set showed good discrimination, and the areas under the ROC curve were 0.840 (95%CI: 0.773-0.900) and 0.822 (95%CI: 0.678-0.936), respectively(Fig. 3). The calibration curve showed good consistency in the training set and the validation set(Fig. 4). Hosmer-Lemeshow goodness-of-fit test indicated that the model was not significant ($p > 0.05$).

Clinical utility

The decision curves threshold probability exceeded 0.13, the nomogram had clinical net benefit. The clinical impact curve could observe the number of people classified as high risk and the

number of true positives under each threshold probability (Fig. 5).

Prognostic analysis

Selection of prognostic factors for CRO infection

Patients with CRO infection were divided into an improved prognosis group and a poor prognosis group. The risk factors for poor prognosis were screened with Lasso-logistic (Fig. 6A, B). This study developed a prognostic analysis model based on the results of the multi-factor analysis (Table 4). Prognostic analysis showed that the area under the ROC curve was 0.817 (95%CI: 0.729-0.893), and the calibration curve fitted well ($P > 0.05$) (Fig. 6C, D).

Table 4 Prognostic analysis

Risk factors	β	OR	95%CI	P-value
Hepatobiliary diseases	1.730	5.640	1.360~23.394	0.0172
Indwelling catheter	2.367	10.663	1.792~63.448	0.0093
Length of hospitalization > 20d	-2.419	0.089	0.030~0.261	<0.0001

Abbreviations: OR Odds Ratio, CI Confidence interval

Prognostic risk assessment

X-tile was used to group the risk values and the KM curve was used for evaluation(Fig. 6E). The results showed that the survival curves of the high- and low-risk groups were separated, and the prognosis of the low-risk group was better ($P<0.0001$).

Fig. 6 (A) Lasso regression model: variable coefficient distribution. (B) Lasso regression model: 10-fold cross-validation, the optimal lambda was 0.116 and the log lambda was -2.156. (C) Prognostic analysis model discrimination. (D) Prognostic analysis model calibration. (E) Survival curve after stratification. Abbreviations: LASSO A least absolute shrinkage and selection operator.

Discussion

Medical technology has developed rapidly in recent years, but the problem of bacterial resistance is still the focus of attention. The United Nations also lists bacterial resistance as an important

health issue, The infection of MDROs in the world is on the rise, and CRO infection has become the main cause of hospital infection in recent years[12-17]. WHO indicates that due to the widespread use of carbapenems and other drugs, 10 million people will die each year by 2050, and the investment cost will need at least 10 billion dollars[18]. A report from Europe evaluated the burden caused by drug-resistant bacteria, Infectious diseases caused 75% of the disease burden, 39% of which came from bacterial infections resistant to first-line antibiotics such as carbapenems, the burden caused by the death of drug-resistant bacteria was equivalent to the sum of the burden caused by influenza, tuberculosis, and AIDS[3]. Livorsi et al. [15] stated that 29,300 people in every 100,000 people in the United States have CRE infection, the detection rate of CRKP in ICU wards in some areas was as high as 72.6%[19]. The CRO strain has a strong ability to spread drug resistance, and its drug resistance mechanism is mainly the production of carbapenemase, abnormal expression of porin combined with ESBLs and AmpC, overexpression of efflux pumps, and conformational changes of the binding site which has mutated drug binding site gene[20, 21].

CRO infection has a poor prognosis due to limited treatment, so it is particularly important to identify the potential risk factors for the prognosis of infection. Previous studies have shown that the history of antimicrobial exposure, the combination of multiple underlying diseases, ICU admission history, immunocompromise, invasive procedures, and related critical illness scores are high-risk factors for the prognosis of CRO infection[22-25]. Therefore, this study collected clinically relevant data from elderly CRO-infected patients and analyzed potential high-risk factors.

A total of 93 old CRO infection strains were included in this study, mainly CRAB, CRPA, and CRKP. The specimen type was mainly sputum, and most of them had been admitted to the ICU.

Baseline data results indicated that CRO patients had more types of drugs, lower immune status, and more invasive procedures than CSO patients, which are consistent with Marquez et al. [26] and Zhao et al. [27].

We developed and validated a model of nosocomial infection of CRO in elderly patients, including respiratory diseases, mechanical ventilation, indwelling urinary catheter, and APACHE II score over 20. Both the training set and the validation set showed good discrimination and consistency. The decision curve and influence curve are proof of the good clinical utility of the model.

Respiratory diseases are one of the important causes of infection and death in the elderly. The elderly have atrophy of the respiratory tract mucosa, decreased secretory function, and visceral tracheal function decline. The above-mentioned reasons make the respiratory function weakened, and the intake and exhaust volume are reduced accordingly, prone to respiratory diseases such as pneumonia, chronic bronchitis, asthma, and tuberculosis; In this study, the sputum was mainly detected, and more strains of CKAB and CRPA were detected. Due to impaired airway structure and function in patients with respiratory diseases, airway self-cleaning and self-purification function decreased, and it was easier to form mucous membranes for long term colonization of the respiratory tract [28-31].

Mechanical ventilation and indwelling urinary catheter are common clinical treatments. The former effectively relieves respiratory failure and gives respiratory support, the latter effectively treats patients with urinary retention, urinary incontinence, or surgery. But the respiratory tract and urethra communicate with the outside world which can easily cause bacterial colonization and infection. Previous studies reported that mechanical ventilation could cause the intubation

membrane to fall off, and the bacteria that form the membrane spread to the lower respiratory tract to cause infection, so there were many reports of ventilator-associated pneumonia[32-34]. Da Silva et al. [35] indicated that invasive operations such as indwelling catheters were significant factors for infection and colonization of multidrug-resistant bacteria. APACHEII score is a measure of the criticality of the patient's condition which quantifies three indicators of the body's acute physiology, age, and chronic health. A score over 20 is considered a risk factor for CRO infection by recording the worst value of the patient within 48 hours of admission. The higher the patient's score at admission, the more critical the condition and the poorer the ability to resist bacterial infections, the more likely it was that patients would get carbapenem-resistant transmission[36].

Further analysis of prognosis, adverse prognostic risk factors included hepatobiliary diseases, indwelling urinary catheters, and hospital stays longer than 20 days. The risk assessment showed that the prognosis of the lower-risk group was lower in the high-risk group ($P < 0.0001$). In patients with hepatobiliary diseases, the function of the macrophage system, especially Kupffer cells, is reduced, because bacteria can enter the abdominal cavity through the intestinal wall, enter the bloodstream and collateral circulation of the intestinal submucosal lymphatics, and cause peritonitis and bacteremia. When combined with CRO infection, a variety of serious comorbidities such as ascites, hepatorenal syndrome, and hepatic coma are induced to form a vicious circle leading to aggravation of the condition[37-39]. Lo E et al. [40] stated that 70%-80% of urinary tract infections are caused by indwelling urinary catheters, as a common iatrogenic infection, indwelling urinary catheters increased the incidence of bacteremia and sepsis, leading to an increase in the mortality of patients. Inconsistent with previous studies, this study showed that

hospital stays longer than 20 days were protective factors which might be due to the poor physical condition of the elderly. Most of them took conservative treatment, and the hospitalization of elderly patients in our hospital for more than 20 days was relatively more adequate, this situation needed to be expanded to further study[41].

However, the study has also identified several limitations. First, the study was a retrospective analysis with a limited sample size and unavoidable bias. Second, the data was based on a research center with 1600 beds in the eastern region of Heilongjiang Province. Although the model has validated in the training set and the validation set, it also needs further validation from different centers.

Conclusions

In conclusion, we established related models of CRO infection and prognosis in elderly patients. The models show good performance. Carbapenem resistance is still a major threat to public health. Screening of clinical risk factors can better assist the clinical development of relevant prediction scores and further determine the best treatment plan for infection prevention and control in elderly patients.

Table 2 Clinical features and characteristics

		CRO(n=93)	CSO(n=84)	P-value
Baseline characteristics	History of hospital	37 (39.8)	35 (41.7)	0.799
	Length of hospitalization> 20d	46 (49.5)	32 (38.1)	0.128
Antibiotic exposure	Appropriate treatment	83 (89.2)	70 (83.3)	0.251
	Carbapenem	30 (32.3)	17 (20.2)	0.071
	β -lactamase inhibitor	86 (92.5)	62 (73.8)	0.001*

	Third-generation cephalosporin	11 (11.8)	8 (9.5)	0.621
	Fourth generation cephalosporin	9 (9.7)	5 (6.0)	0.359
	Fluoroquinolone	15 (16.1)	10 (11.9)	0.420
	Aminoglycoside	33 (35.5)	13 (15.5)	0.002*
	Antifungal agent	16 (17.2)	2 (2.4)	0.001*
	Antibiotic treatment > 7d	82 (88.2)	64 (76.2)	0.036*
	Antibiotics before infection	92 (98.9)	78 (92.9)	0.093
Length of stay in ICU	Stay time(d)			<0.001*
	0	15 (16.1)	55 (65.5)	
	1—7	32 (34.4)	19 (22.6)	
	> 7	46 (49.5)	10 (11.9)	
Basic disease	Respiratory diseases	88 (94.6)	52 (61.9)	<0.001*
	Diabetes	17 (18.3)	23 (27.4)	0.148
	Malignant tumor	5 (5.4)	6 (7.1)	0.627
	Cardiovascular diseases	69 (74.2)	55 (65.5)	0.206
	Septic shock	5 (5.4)	8 (9.5)	0.291
	Nervous system disease	70 (75.3)	41 (48.8)	<0.001*
	Kidney disease	11 (11.8)	21 (25.0)	0.023*
	Hypoproteinemia	19 (20.4)	11 (13.1)	0.194
	Hepatobiliary diseases	15 (16.1)	17 (20.2)	0.478
	Combine other bacteria	35 (37.6)	17 (20.2)	0.011*

Immunization	Immunosuppressive agent	62 (66.7)	37 (44.0)	0.002*
	Parenteral nutrition	62 (66.7)	51 (60.7)	0.410
	Chemotherapy	1 (1.1)	5 (6.0)	0.169
Intrusive operation	Mechanical Ventilation	60 (64.5)	17 (20.2)	<0.001*
	Indwelling catheter	80 (86.0)	41 (48.8)	<0.001*
	surgery	50 (53.8)	42 (50.0)	0.617
	Central venous catheter	28 (30.1)	15 (17.9)	0.058
	Indwelling dural drainage tube	14 (15.1)	6 (7.1)	0.097
	Chest tube	13 (14.0)	1 (1.2)	0.002*
	Abdominal catheterization	7 (7.5)	19 (22.6)	0.005*
Related ratings	APACHEII score over 20	37 (39.8)	5 (6.0)	<0.001*

* P < 0,05; *Abbreviations*: CRO Carbapenem-resistant organism, CSO Carbapenem-sensitive organism, ICU Intensive care unit, APACHEII Acute Physiology and Chronic Health Evaluation II

237 Abbreviations

238 CRO: Carbapenem-resistant organism; CSO: Carbapenem-sensitive organism; LASSO: A least absolute

239 shrinkage and selection operator; ROC: Receiver operating characteristic; CI: Confidence interval; ESBLs: Extended-spectrum

240 β -lactamase; OR: Odds Ratio; CRE: Carbapenem-resistant Enterobacteriaceae;

241 CRAB: Carbapenem-resistant Acinetobacter baumannii; CRPA: Carbapenem-resistant Pseudomonas

242 aeruginosa; ICU: Intensive care unit; APACHEII: Acute Physiology and Chronic Health Evaluation II;

243 MDROs: Multidrug-resistant organisms

244 Declarations

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247 **Author's contributions**

248 All authors contributed to this work. WY1, LHL and CY collected the data. WY1, FYJ and XJF analyzed the data. WY1 wrote the first
249 draft of the manuscript. WY2 designed this work and critically reviewed the manuscript. All authors reviewed and approved the final
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255 **Availability of data and materials**

256 All data generated or analyzed during this study are included in this manuscript.

257 **Ethics approval and consent to participate**

258 The study protocol was approved by the Ethics Committee of Jiamusi University Clinical Medical College for research. Informed consent
259 was waived because the study was conducted retrospectively.

260 **Consent for publication**

261 Not applicable.

262 **Competing interests**

263 The authors declare that they have no competing interests.

264 **Author information**

265 Yao Wang¹, 986627900@qq.com

266 Huiling Li¹, lihuilingwujian@163.com

267 Ying Chen², 15694549905@163.com

268 Yanjun Fu¹, fuyanjun1981@163.com

269 Jianfeng Xi¹, 1017341923@qq.com

270 Yong Wang¹, jmsuwy@163.com

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Figures

Figure 1

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Figure 2

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