Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Sara Silvershein Date

Predictive Model for Carbapenem Resistant *Pseudomonas aeruginosa* on Admission to Acute Care Hospitals

By

Sara Silvershein
MPH

Epidemiology

_____

Scott Fridkin, MD
Committee Chair

_____

Jessica Howard-Anderson, MD, MSc
Committee Member

Predictive Model for Carbapenem Resistant *Pseudomonas aeruginosa* on Admission to Acute Care Hospitals

By

Sara Silvershein

Bachelor of Science in Public Health
Tulane University
2022

Bachelor of Science
Tulane University
2022

Thesis Committee Chair: Scott Fridkin, MD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2024

Abstract

Predictive Model for Carbapenem Resistant *Pseudomonas aeruginosa* on Admission to Acute Care Hospitals
By Sara Silvershein

Background: Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is a multidrug-resistant bacteria that frequently causes healthcare-associated infections (HAI). Since CRPA cannot be treated with carbapenems, treatment options are limited. CRPA can colonize patients and the healthcare environment, increasing the risk of healthcare-associated transmission and outbreaks. We aim to create a predictive classification model to determine CRPA carriage on admission to an acute care hospital.

Methods: We performed a case-control study using electronic medical record (EMR) data from 4 healthcare facilities within a single healthcare system from 1/1/2014 to 12/31/2021. Cases were defined as adult patients (>18 years old) with their first inpatient encounter where a clinical culture identified *P. aeruginosa* resistant to meropenem or imipenem collected on the day prior to admission or within the first 2 hospital days. Only the first CRPA culture from patients was included. Controls included admissions from the same hospital during the same month and year that the case sample was collected. the dataset was split into training and testing datasets. Univariable logistic regression was used to determine crude associations between the model covariates and the outcome. Best subset selection was used to select the final model. A multivariate logistic regression model and ten-fold cross validation were used to estimate coefficients for predictors of interest in the model. The models were assessed by receiver operating characteristic (ROC) curves and area under the curve (AUC). A secondary analysis was conducted to exclude patients with cystic fibrosis using the same methods as the primary model.

Results: We identified 521 cases and 560,623 controls. The AUC of the primary model was 0.80. The AUC of the secondary model was 0.72. Cystic fibrosis was the most significant predictor of CRPA. Diabetes as well as days of carbapenem treatment, infection diagnosis, and ventilation in the last year were significant predictors of CRPA carriage independent of cystic fibrosis.

Conclusion: We developed a model with good predictive ability. Implementation of this model in healthcare facilities could help with earlier identification of patients with CRPA and decrease the risk of healthcare-associated transmission. Future studies could prospectively validate this model's performance to accurately identify patients with CRPA carriage on admission.

Predictive Model for Carbapenem Resistant *Pseudomonas aeruginosa* on Admission to Acute Care Hospitals

By

Sara Silvershein

Bachelor of Science in Public Health
Tulane University
2022

Bachelor of Science
Tulane University
2022

Thesis Committee Chair: Scott Fridkin, MD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2024

Table of Contents

Background and Public Health Relevance

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is a multidrug-resistant bacteria, defined as being resistant to the carbapenem class of antibiotics including meropenem, imipenem, and doripenem. CRPA has been classified as a serious public health threat by the CDC[1]. *P. aeruginosa* frequently causes healthcare-associated infections (HAI) in the United States and around the world[1]. Since CRPA cannot be treated with carbapenems, there are very few and sometimes no other available antibiotic options for patients infected with CRPA[2].

Carbapenem resistance is acquired in *P. aeruginosa* through chromosomal mutations and the horizontal transfer of carbapenemase genes. Carbapenemase genes can be transferred to *P. aeruginosa* from other gram-negative bacteria. In the United States, between 2011-2020, the percentage of *P. aeruginosa* healthcare associated infections (HAIs) that were carbapenem resistant decreased from 20% to 13%[3]. In 2021 there was a slight increase to 14%[3], but it is too early to know if this is a concerning trend that will continue. The percentage of *P. aeruginosa* isolates resistant to carbapenems in Georgia has, historically, been higher than the national average; In 2021, 18% of *P. aeruginosa* isolates tested in Georgia were resistant to carbapenems[3].

CRPA can colonize patients and the healthcare environment, increasing the risk of healthcare-associated transmission and outbreaks. *P. aeruginosa* is capable of surviving on surfaces and in water systems for hours to months at a time due to mechanisms like biofilm formation[4]. Implementing contact precautions, which requires healthcare personnel to wear gowns and gloves when caring for patients, is frequently used for patients with CRPA and may reduce the risk of healthcare-associated transmission. Identifying patients who are at a high risk for infection or colonization (“carriage”) with CRPA on admission can allow healthcare facilities

to empirically use contact precautions and/or selectively test those patients for CRPA. Once a patient is identified to have CRPA carriage, enhanced protocols may include the implementation of contact precautions and enhanced cleaning of sinks, toilets, and other wastewater plumbing[1].

Predictive modeling is one method of identifying patients at high risk for CRPA carriage. Individuals with cystic fibrosis are especially vulnerable to CRPA. Additional patient level risk factors previously associated with CRPA include age, hospitalization in the last year, diabetes, prior admission to an ICU, needing a ventilator, previous diagnosis of an infection, and increased exposure to antibiotics[5-8]. Prior CRPA modeling studies have primarily focused on the ICU population and less is known about patients being admitted to the hospital who are not critically ill. In this study, we aim to evaluate risk factors for CRPA carriage for all patients hospitalized at an academic healthcare system in Georgia.

Methods

We performed a case-control study using electronic medical record (EMR) data from 4 healthcare facilities within a single healthcare system from 1/1/2014 to 12/31/2021. Cases were defined as adult patients (≥18 years old) with their first inpatient encounter where a clinical culture identified *P. aeruginosa* resistant to meropenem or imipenem collected on the day prior to admission or within the first 2 hospital days. Doripenem susceptibility was not routinely tested or reported, and thus was not included in the case definition. A *P. aeruginosa* isolate was defined as resistant to meropenem or imipenem based on Clinical and Laboratory Standards Institute (CLSI) defined breakpoints (MIC of ≥8 μg/ml)[9]. Only the first CRPA culture from patients was included. Controls included admissions from the same hospital during the same month and year that the case sample was collected. The four hospitals included in the study vary in size and

patient population. Hospital A and Hospital B are large, primarily academic hospitals with over 500 beds each. Hospital C and Hospital D are smaller 100-150 bed community hospitals.

Model predictors were selected for consideration in the model based on literature review. Data on gender, race, ethnicity, hospital, age, total length of stay, previous carbapenem use, previous ICU admission, total days of antibiotic therapy, number of previous hospitalizations, previous ventilation, and culture source was abstracted from the EMR. Non-demographic variables were restricted to the year prior to admission. Administrative billing codes (ICD-9 and ICD-10) were used to determine if the patient had a diagnosis of cystic fibrosis, diabetes, or previous infection.

To develop the predictive model, the dataset was split into two, a training (~80%) and a testing (~20%) dataset. Assignment to the training or testing sets was determined by stratified random sampling. Univariable logistic regression was used to determine crude associations between the model covariates and the outcome. Best subset selection was used to select the final, most parsimonious model. A multivariate logistic regression model was used to estimate coefficients for predictors of interest in the model. Ten-fold cross validation was conducted to reduce the likelihood of overfitting and tune the model parameters. Adjusted odds ratio and corresponding confidence intervals were calculated and reported to determine the significance of the model covariates. The trained model was assessed using Area Under the Curve (AUC). The predictive ability of the trained model was tested with unseen data from the testing dataset and assessed by using receiver operating characteristic (ROC) curves and AUC.

A secondary analysis was conducted to exclude patients with cystic fibrosis due to the strong association between CRPA and cystic fibrosis. First, the model trained with the cystic fibrosis patients was tested with the training population without cystic fibrosis patients. Second,

a new model was trained excluding the cystic fibrosis patients. We used the same methods for model selection and validation as the original model. The new model was then tested using the same unseen test data as the original model.

All statistical analysis was completed using R statistical software and RStudio. Model selection was completed using the leaps package and model training and cross validation was completed using the caret package. This study was approved by the Emory University Institutional Review Board.

Results

From 2014–2021, we identified 521 cases and 560,623 controls. The training dataset contained 409 cases and 448,515 controls. Cases and controls had similar demographics, however, cases were more likely to be males (OR 1.89, 95% CI 1.56, 2.31) (Table 1). The most common culture sources of CRPA were respiratory (40%) and urine (33%) (Table 1).

Univariable logistic regression demonstrated that all selected non-demographic predictors were independently associated with being a case (Table 1). These include cystic fibrosis (OR 159.00, 95% CI 121.00, 205.00), length of stay in the previous year (OR 1.02, 95% CI 1.02, 1.02), carbapenem days of therapy in the prior year (OR 1.08, 95% CI 1.07, 1.09), diabetes (OR 1.89, 95% CI 1.54, 2.30), ICU admission in the previous year (OR 5.04, 95% CI 3.85, 6.49), total days of antibiotic therapy in the previous year (OR 1.01, 95% CI 1.01, 1.01), number of hospitalizations in the previous year (OR 1.15, 95% CI 1.11, 1.18), infection diagnosis in the previous year (OR 7.24, 95% CI 5.95, 8.80), and requiring mechanical ventilation in previous year (OR 13.8, 95% CI 10.0, 18.5) (Table 1).

The multivariable logistic regression model included cystic fibrosis and diabetes, as well as the following healthcare exposures from the 365 days before admission: total length of stay,

days of carbapenem treatment, ICU admission, number of previous hospitalizations, infection diagnosis, and previous ventilation. All variables in multivariable model were independently associated with being a case except for the total length of stay in the prior year (1.01, 95% CI 1.00, 1.02) (Table 2). Cystic fibrosis was the strongest predictor of CRPA (aOR 89.3, 95% CI 65.6, 121). Previous ventilation (aOR 4.61, 95% CI 2.94, 7.20) and a prior infection diagnosis in the last year (aOR 3.69, 95% CI 1.37, 2.08) were also strong associated with CRPA. The AUC for the model was 0.80 when run with the unseen data in the testing dataset (Figure 1).

In our secondary analysis, we aimed to evaluate model performance excluding patients with cystic fibrosis. First, we removed cystic fibrosis patients from the training dataset. We then applied the original model to the training dataset with cystic fibrosis patients excluded, and the model AUC was reduced to 0.76. Next, we created, a new, optimized model that included length of stay in the previous year, previous carbapenem days of therapy, diabetes, previous ICU admission, number of previous hospitalizations, previous infection diagnosis, and previous ventilation. All healthcare exposures were from the year prior to admission. The most impactful predictor of CRPA identified by the secondary model was previous infection diagnosis (aOR 5.07, 95% CI 4.12, 6.23) (Table 3). Previous ventilation (aOR 4.47, 95% CI 2.92, 6.79), previous carbapenem treatment (aOR 1.05, 95% CI 1.03, 1.06) and diabetes (aOR 1.49, 95% CI 1.22, 1.83) were also significantly associated with CRPA (Table 3). Length of previous stay (aOR 1.01, 95% CI 1.00, 1.01), previous ICU admission (aOR 1.21, 95% CI 0.82, 1.73) and number of previous hospitalizations (aOR 1.02 95% CI 0.961, 1.08) were not significant in this model (Table 3). The AUC of the retrained model was 0.73. After running the retrained model with the testing dataset, which contained cystic fibrosis patients, the AUC was 0.72 (Figure 2).

Discussion

CRPA is found in the environment but is more prevalent in the hospital setting. We examined numerous covariates related to previous healthcare exposures to build a model that can predict a patient's likelihood of having CRPA on admission. Having a previous infection in the last year was a significant predictor of CRPA in these models. It was independently associated with the outcome in the univariate analysis and remained significant in both models. Similarly, if a patient required mechanical ventilation in the prior year their odds of having CRPA was almost five times that of someone who had not required mechanical ventilation after adjusting for other risk factors. This was the only covariate included in the model that was related to an indwelling device, which have been previously associated with CRPA in the literature.

Total length of stay and number of previous hospitalizations both quantify a patients' prior healthcare exposure. By including both variables as covariates, we tried to answer if a longer stay in a hospital and/or if multiple independent admissions have an association with CRPA. Both variables were selected for inclusion in the primary and secondary models. Total length of stay was not significant in either model. Number of hospitalizations in the previous year was a risk factor in the univariable analysis. The adjusted odds ratio for number of previous hospitalizations was determined to be a protective factor rather than a risk factor in the primary model. Number of hospitalizations was significant in the primary model but was not a significant risk factor in the secondary model. Further research is necessary to determine the true relationship with CRPA and number of hospitalizations. In our dataset, all instances of a person being admitted to healthcare facility more than 13 times in the previous year were associated with the controls. In addition to time spent in a hospital and number of admissions, being admitted to an ICU was a significant in the univariate analysis. Previous admission to an ICU

was included in both models but was not significant in the model trained without cystic fibrosis patients.

Antibiotics usage is an important risk factor to consider for multidrug resistant organisms. Total days of antibiotic therapy was identified as a possible risk factor during the literature review but was not selected for inclusion in either model. The unadjusted odds ratio was very close to the null. Previous carbapenem treatment, a more specific variable looking at length of antibiotic treatment, was selected for inclusion in both models. The significance of the carbapenem variable in both models, and the exclusion of the generic antibiotic therapy variable, indicates that the use of broad-spectrum antibiotics like carbapenems is more likely increase a patient's risk for CRPA than other antibiotic use. Inclusion of additional antibiotic classes in the future could provide more insight.

Diabetes was risk factor identified in the literature for potential inclusion in the model and was selected for inclusion in the primary and secondary models. The models showed that diabetes is associated with CRPA independent of the cystic fibrosis diagnosis. The inclusion and significance of diabetes in the secondary model is important because cystic fibrosis related diabetes (CFRD) is a common complication of cystic fibrosis in adults. The diabetes variable may include patients with CFRD. There is no ICD-9 or ICD-10 code for CFRD. When we excluded individuals with cystic fibrosis from the secondary model, we eliminated a potential source of confounding between diabetes and CRPA carriage.

Cystic fibrosis was the most significant risk factor in the model. Of the risk factors included in the models, cystic fibrosis had the highest crude odds ratio (OR 159, 95% CI 121, 205). Once adjusted for the other covariates in the model, the odds ratio for cystic fibrosis remained high (aOR 89.3, 95% CI 65.6, 121). The analysis of the first model showed an

overwhelming association between cystic fibrosis and CRPA carriage. This association may have skewed the model and led to an incomplete understanding of the other predictors in the model, especially for patients with cystic fibrosis. The covariates selected for this model included the following the year prior to admission: length of stay, days of carbapenem treatment, ICU admission, number of hospitalizations, previous infection, and ventilation. The secondary model also included diabetes as a covariate. Despite an 18% reduction in the number of cases available to train the model, the secondary model produced similarly significant odds ratios to the original model but a lower AUC. The models in this study provide evidence that previous carbapenem treatment, diabetes, previous infection diagnosis, and previous ventilation are significant predictors of CRPA carriage independent of a patient's cystic fibrosis status.

A limitation of this study is that we were not able to capture information on healthcare encounters and treatments that occurred outside of the healthcare system in this study. Data on previous carbapenem treatment, length of stay, total days of antibiotic therapy, ICU admission, previous hospitalizations, and previous ventilation in the last year were collected from billing codes and records from a singular healthcare system in a large metropolitan area. It is possible that cases and controls sought care and received treatment at other healthcare facilities in the year before the admission used in this study, but this information would not be available in our dataset. Therefore, the values of our predictors could be an underestimation of the healthcare utilized by our patient population. Additionally, the large imbalance between cases and controls in this dataset could be affecting the outcome of the analysis. CRPA is a rare event and cases represent approximately 0.9% of the training dataset. Measures like stratified random sampling were required to ensure appropriate representation in both the training and testing datasets. The imbalance is large enough that it could be affecting the performance of the model and biasing the

model towards the controls. Future studies with improved balance between cases and controls may yield different results.

Many of the existing predictive models for CRPA in the literature aim to predict mortality or the likelihood of carbapenem resistance in patients with *P. aeruginosa* infections. There is little prior work developing a predictive model to identify CRPA among all hospitalized populations on admission. This model could be used as a tool for acute care healthcare facilities and, in the future, could be integrated to EMRs. By identifying high-risk patients on admission, protocols can be put in place to limit the transmission of CRPA to the environment and to healthcare workers. Even if a patient isn't screened for CRPA, a flag in the EMR as a potential high-risk patient could provide situational awareness and possibly better adherence to standard precautions.

Conclusion

CRPA is a serious public health threat that causes a significant number of healthcare-associated infections in the United States and globally. Patients with CRPA carriage, which includes patients that are colonized and infected with CRPA, can transmit the bacteria to the environment which increases the risk of healthcare associated transmission to other patients. In our multivariable analysis, the most significant risk factor for CRPA on admission to acute care facilities was cystic fibrosis. Previous carbapenem treatment, diabetes, previous infection diagnosis, and previous ventilation were also significant predictors of CRPA carriage independent of cystic fibrosis. Implementation of this model in healthcare facilities could help with earlier identification of patients with CRPA and decrease the risk of healthcare-associated transmission. Future studies could prospectively validate this model's performance to accurately identify patients with CRPA carriage on admission.

Table 1: Patient Characteristics and Univariable Analysis

Characteristics	Cases[1] N=409	Controls[1] N=448,515	Unadjusted OR	95% CI	p
Gender					
Female	164 (40%)	250,720 (56%)	ref	--	--
Male	245 (60%)	197,785 (44%)	1.89	1.56, 2.31	<0.001
Unknown	0 (0%)	1 (<0.1%)			
Race					
Black	150 (37%)	168,939 (38%)	ref	--	--
White	201 (49%)	197,303 (44%)	1.15	0.93, 1.42	0.2
Other[3]	13 (3.2%)	16,711 (3.7%)	0.88	0.47, 1.48	0.6
Unknown	45 (11%)	65,553 (15%)			
Ethnicity					
Non-Hispanic	348 (85%)	358,333 (80%)	1.79	0.92, 4.19	0.13
Hispanic	7 (1.7%)	12,893 (2.9%)	ref	--	--
Unknown	54 (13%)	77,280 (17 %)			
Age (years)	59 (42, 71)	60 (43, 72)	1.00	0.99, 1.00	0.2
Cystic Fibrosis	75 (18%)	633 (0.1%)	159	121, 205	<0.001
Total Length of Stay (days)[2]	7 (0, 26)	0 (0, 4)	1.02	1.02, 1.02	<0.001
Previous Carbapenem Days of Therapy[2]	0 (0, 4)	0 (0, 0)	1.08	1.07, 1.09	<0.001
Diabetes	155 (38%)	109,737 (24%)	1.89	1.54, 2.30	<0.001
Previous ICU Admission[2]	68 (17%)	17,074 (3.8%)	5.04	3.85, 6.49	<0.001
Total Days of Antibiotic Therapy[2]	8 (0, 40)	0 (0,0)	1.01	1.01, 1.01	<0.001
Number of Previous Hospitalizations[2]	1 (0, 2)	0 (0, 1)	1.15	1.11, 1.18	<0.001
Previous Infection[2]	180 (44%)	43,907 (9.8%)	7.24	5.95, 8.80	<0.001
Previous Ventilation[2]	47 (11%)	4,195 (0.9%)	13.8	10.0, 18.5	<0.001
Culture Source					
Respiratory	207 (40%)				
Urine	173 (33%)				
Wound	45 (8.6%)				
Blood	21 (4.0%)				
Other	69 (13%)				
Not Specified	6 (1.2%)				
Hospital					
Hospital A	224 (55%)	162,587 (36%)	ref	--	--
Hospital B	84 (21%)	143,831 (32%)	0.420	0.330, 0.540	<0.001
Hospital C	68 (17%)	97,651 (22%)	0.510	0.338, 0.660	<0.001
Hospital D	33 (8.1)	44,437 (9.9%)	0.540	0.370, 0.770	<0.001

[1]Median (IQR) or n (%) [2]In the 365 days prior to admission

[3]Other race includes Asian, Native Hawaiian or Pacific Islander, American Indian or Alaskan Native, and Multiple Race

Table 2 Primary Multivariable Model

Characteristics	aOR	95% CI
Cystic Fibrosis	89.3	65.6, 121
Total Length of Stay[1]	1.01	1.00, 1.02
Previous Carbapenem Days of Therapy[1]	1.04	1.02, 1.05
Diabetes	1.69	1.37, 2.08
Previous ICU Admission[1]	1.53	1.03, 2.23
Number of Previous Hospitalizations[1]	0.930	0.873, 0.986
Previous Infection[1]	3.69	2.97, 4.58
Previous Ventilation[1]	4.61	2.94, 7.20

[1]In the 365 days prior to admission

Figure 1 ROC Curve for Primary Multivariable Model

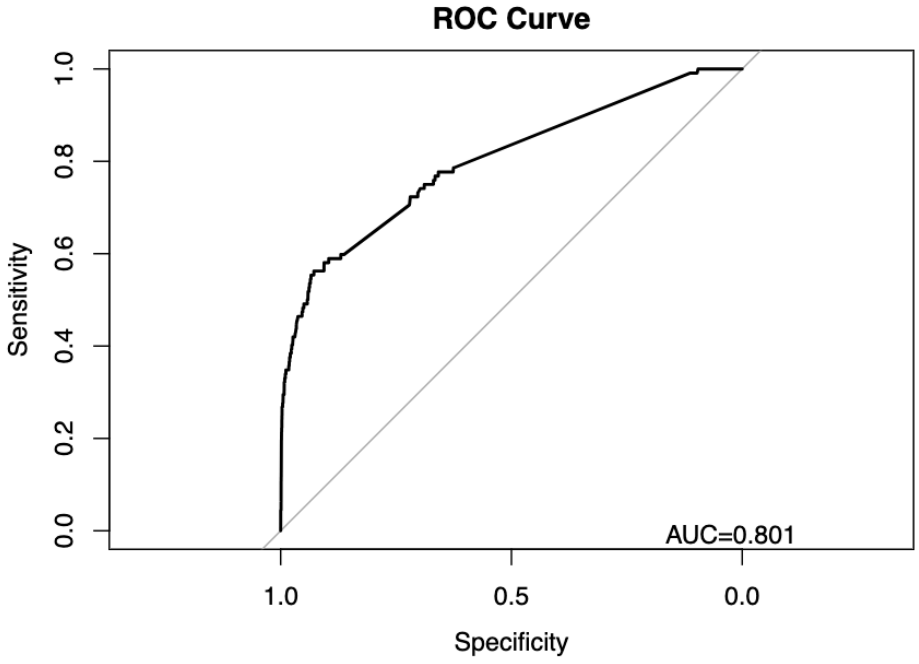
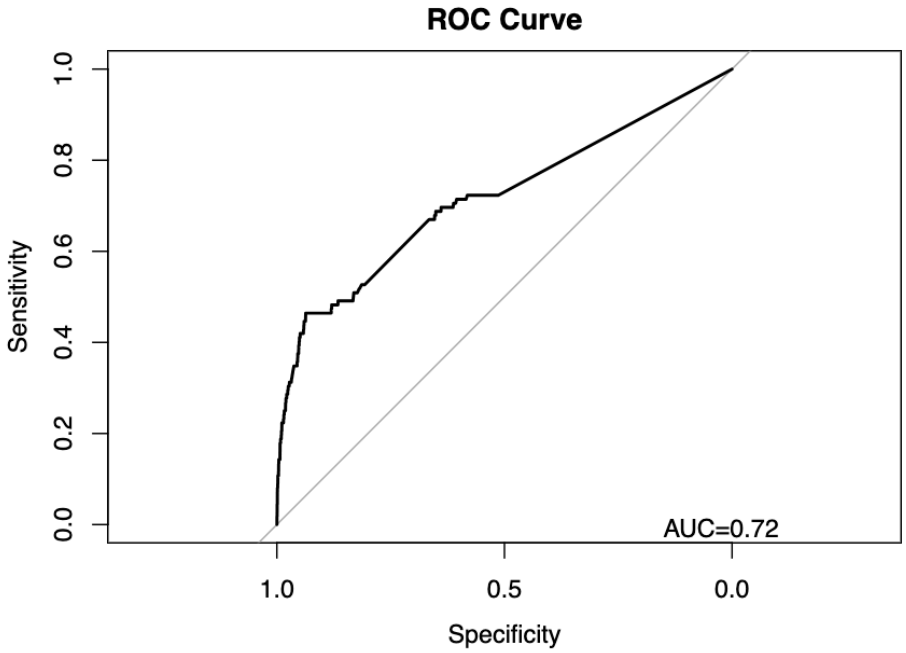


Table 3 Results for Secondary Analysis: Model Trained without Cystic Fibrosis Patients

Characteristics	aOR	95% CI
Total Length of Stay[1]	1.01	1.00, 1.01
Previous Carbapenem Days of Therapy[1]	1.05	1.03, 1.06
Diabetes	1.49	1.22, 1.83
Previous ICU Admission[1]	1.21	0.82, 1.73
Number of Previous Hospitalizations[1]	1.02	0.961, 1.08
Previous Infection[1]	5.07	4.12, 6.23
Previous Ventilation[1]	4.47	2.92, 6.79

[1]In the 365 days prior to admission

Figure 2 ROC Curve for Secondary Analysis: Model Trained without Cystic Fibrosis Patients



References:

1. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA2019.
2. Reyes J, Komarow L, Chen L, et al. Global epidemiology and clinical outcomes of carbapenem-resistant Pseudomonas aeruginosa and associated carbapenemases (POP): a prospective cohort study. The Lancet Microbe 2023;4(3):e159-e170. DOI: 10.1016/S2666-5247(22)00329-9.
3. Centers for Disease Control and Prevention. Antimicrobial Resistance & Patient Safety Portal. (https://arpsp.cdc.gov/profile/antibiotic-resistance/carbapenem-resistant-pseudomonas-aeruginosa?year-select-resistance-by-state=year300&year-select-resistance-by-state-and-region=year301).
4. Moradali MF, Ghods S, Rehm BH. Pseudomonas aeruginosa Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. Front Cell Infect Microbiol 2017;7:39. (In eng). DOI: 10.3389/fcimb.2017.00039.
5. Thuong M, Arvaniti K, Ruimy R, et al. Epidemiology of Pseudomonas aeruginosa and risk factors for carriage acquisition in an intensive care unit. J Hosp Infect 2003;53(4):274-82. (In eng). DOI: 10.1053/jhin.2002.1370.
6. Walters MS, Grass JE, Bulens SN, et al. Carbapenem-Resistant Pseudomonas aeruginosa at US Emerging Infections Program Sites, 2015. Emerg Infect Dis 2019;25(7):1281-1288. (In eng). DOI: 10.3201/eid2507.181200.
7. Zhang D, Cui K, Wang T, et al. Risk factors for carbapenem-resistant Pseudomonas aeruginosa infection or colonization in a Chinese teaching hospital. J Infect Dev Ctries 2018;12(8):642-648. (In eng). DOI: 10.3855/jidc.10150.

8. Hu Y, Qing Y, Chen J, et al. Prevalence, Risk Factors, and Molecular Epidemiology of Intestinal Carbapenem-Resistant Pseudomonas aeruginosa. Microbiol Spectr 2021;9(3):e0134421. (In eng). DOI: 10.1128/Spectrum.01344-21.

9. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 34 ed: Clinical and Laboratory Standards Institute; 2024:416.