Infection and Drug Resistance

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ORIGINAL RESEARCH

Artificial Intelligence-Clinical Decision Support System in Infectious Disease Control: Combatting Multidrug-Resistant *Klebsiella pneumoniae* with Machine Learning

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Purpose: The World Health Organization has identified *Klebsiella pneumoniae* (KP) as a significant threat to global public health. The rising threat of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) leads to prolonged hospital stays and higher medical costs, necessitating faster diagnostic methods. Traditional antibiotic susceptibility testing (AST) methods demand at least 4 days, requiring 3 days on average for culturing and isolating the bacteria and identifying the species using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), plus an extra day for interpreting AST results. This lengthy process makes traditional methods too slow for urgent clinical situations requiring rapid decision-making, potentially hindering prompt treatment decisions, especially for fast-spreading infections such as those caused by CRKP. This research leverages a cutting-edge diagnostic method that utilizes an artificial intelligence-clinical decision support system (AI-CDSS). It incorporates machine learning algorithms for the swift and precise detection of carbapenem-resistant and colistin-resistant strains.

Patients and Methods: We selected 4307 KP samples out of a total of 52,827 bacterial samples due to concerns about multi-drug resistance using MALDI-TOF MS and Vitek-2 systems for AST. It involved thorough data preprocessing, feature extraction, and machine learning model training fine-tuned with GridSearchCV and 5-fold cross-validation, resulting in high predictive accuracy, as demonstrated by the receiver operating characteristic and area under the curve (AUC) scores, laying the groundwork for our AI-CDSS.

Results: MALDI-TOF MS analysis revealed distinct intensity profiles differentiating CRKP and susceptible strains, as well as colistin-resistant *Klebsiella pneumoniae* (CoRKP) and susceptible strains. The Random Forest Classifier demonstrated superior discriminatory power, with an AUC of 0.96 for detecting CRKP and 0.98 for detecting CoRKP.

Conclusion: Integrating MALDI-TOF MS with machine learning in an AI-CDSS has greatly expedited the detection of KP resistance by approximately 1 day. This system offers timely guidance, potentially enhancing clinical decision-making and improving treatment outcomes for KP infections.

Keywords: carbapenem, colistin, diagnostic accuracy, antibiotic stewardship, MALDI-TOF MS

Introduction

The World Health Organization has identified *Klebsiella pneumoniae* (KP) as a significant threat to global public health.[1] The global health landscape is currently confronting an unparalleled challenge due to the surge in antibiotic-resistant bacteria, particularly underscored by the emergence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP).[2,3] Globally,

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the prevalence of CRKP in KP infections is approximately 29%, with regional disparities spanning from 14% to 66%.[4] These strains, resistant to our most crucial antibiotics, significantly hinder the efforts in managing infections, especially within hospital environments, thereby intensifying the incidence of nosocomial infections.[3,5,6] These infections are linked to increased morbidity and mortality, with death rates between 37.2% and 42.1%, and they contribute to prolonged hospital stays and higher medical costs, exacerbating their economic and healthcare impacts.[7–9] This situation urgently calls for the creation of novel strategies for both the management and treatment of infections attributed to these resistant microbes.[10,11]

The advent of colistin as a therapeutic option for infections caused by multidrug-resistant bacteria was initially met with optimism.[5,6] Nonetheless, the rapid onset of resistance to colistin, including the phenomenon of heteroresistance among CRKP strains, has swiftly underscored the inadequacies inherent in existing treatment modalities and diagnostic approaches.[12,13] The swift adaptation and resistance evolution of these bacterial pathogens necessitate an urgent reassessment and enhancement of our current diagnostic and therapeutic strategies.[10] Traditional methods used for antibiotic susceptibility testing (AST) in current practice demand at least 4 days. Approximately 3 days on average are required for culturing and isolating the bacteria, and thereafter identifying the species using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS); an extra day is required for interpreting the AST results. This lengthy process makes traditional methods too slow for urgent clinical situations requiring rapid decision-making, potentially hindering prompt treatment decisions, especially for fast-spreading infections like those caused by CRKP.[10]

In light of this pressing need, there is a collective agreement on the urgency to develop and deploy diagnostic technologies that can accurately keep pace with the rapidly evolving dynamics of antibiotic resistance.[10] The advent of innovative diagnostic solutions, particularly the integration of MALDI-TOF MS with complex data analysis algorithms, stands as a beacon of hope.[14,15] Such advancements promise to transform our capabilities in detecting resistant strains swiftly, thus enabling more precise and efficacious interventions.[16] By analyzing protein expression profiles, techniques like MALDI-TOF MS can rapidly identify bacterial strains; when integrated with artificial intelligence (AI) for interpreting complex molecular data, this combination significantly enhances diagnostic capabilities, reducing the identification time of resistant strains from a day to minutes, thereby enabling prompt, targeted interventions.[16]

Building on these diagnostic improvements, our research focused on creating an AI-clinical decision support system (AI-CDSS) to enhance decision-making for carbapenems and colistin in treating KP infections. Antibiotic resistance could be predicted by AI-CDSS in minutes using MALDI-TOF MS protein profiles, compared to the traditional AST method that requires one day, thus providing rapid antibiotic recommendations. This system aims to improve patient care and effectively combat antibiotic resistance.

Materials and Methods

Study Designs and Data Collection

From January to December 2022, data collection was conducted at Tri-Service General Hospital, a major urban tertiary center, and four secondary hospitals. While the medical center caters to diverse patient care needs, the secondary hospitals primarily manage chronic diseases. Three of these secondary hospitals are located in the northern region, and one is situated on an island of our country, addressing different geographic and demographic conditions. Culture specimens, including blood, urine, throat swabs, sputum, body fluids, wound swabs, and catheters, were collected using standard sterile techniques from patients suspected of bacterial infections. These samples were promptly transported to the microbiology laboratory for further analysis. In total, 52,827 bacterial samples were collected, and KP was detected in 4307 samples.

Species identification took, on average, 3 days, including initial culturing of specimens on non-selective media, followed by transfer to selective media to isolate colonies, and finally, identification using MALDI-TOF MS (bioMérieux, France). Subsequently, AST interpretation took an additional day using VITEK-2 cards, which included bacterial growth control wells to ensure accurate antibiotic responses and reduce potential human or instrumental errors. We analyzed three antibiotics: colistin, doripenem (DOR), and imipenem (IPM), with susceptibility ranges of 0.5–16 μg/

mL, 0.12–8 μg/mL, and 0.25–16 μg/mL, respectively. Stringent quality control was maintained with recommended strains, including *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ssp. pneumoniae ATCC 700603. The susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints. The quality control results for these three antibiotics must fall within the recommended ranges specified by the CLSI M100 guidelines; any results outside these ranges indicate unsatisfactory quality control.[17]

The primary objective of this study was twofold: first, to distinguish whether the strains were CRKP, and second, to determine if they were colistin-resistant *Klebsiella pneumoniae* (CoRKP). The study design and flowchart of this study are detailed in Figure 1.

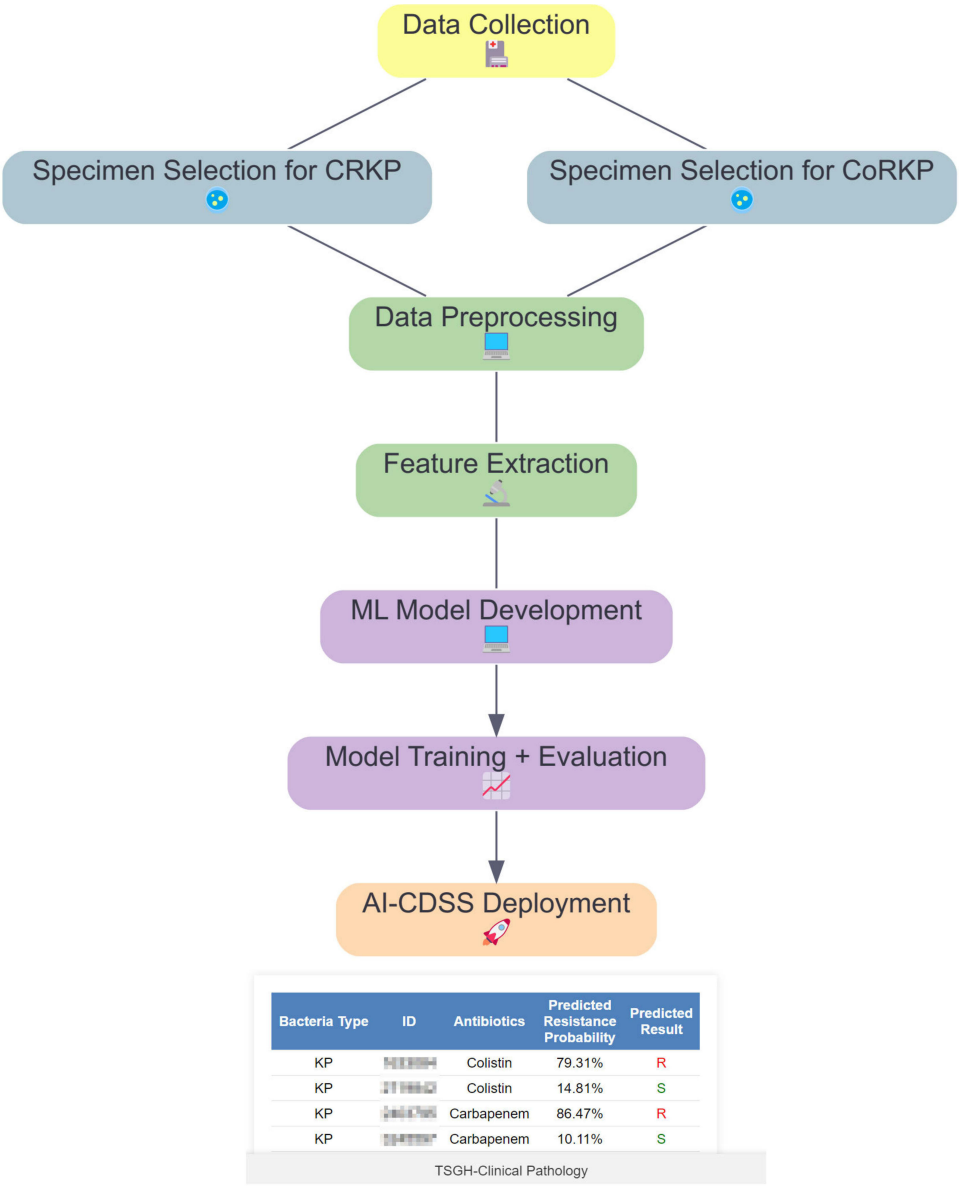

Figure 1 Flowchart of the study design. Commencing with the study focus and progressing to data collection. The pathway splits into specimen selection for CRKP (carbapenem-resistant *Klebsiella pneumoniae*) and colistin-resistant *Klebsiella pneumoniae* (CoRKP). Following this, data preprocessing and feature extraction take place, pinpointing vital m/z ratio segments for the development of a machine learning model. This model undergoes training and evaluation to ensure its accuracy and reliability. The final step is the deployment of the artificial intelligence-clinical decision support system (AI-CDSS). The table on the web page shows example outputs, including bacteria type, patient ID, antibiotics tested, predicted resistance probability, and final prediction ("R" for resistant or "S" for susceptible).

Specimen Selection for the CRKP Training Model

In our study, CRKP was defined based on resistance to either IPM or DOR, categorized as "Resistant" or "Intermediate" in antimicrobial susceptibility. Our hospital monitors CRKP trends and regularly updates treatment protocols, integrating quarterly reviews of the CRKP definition from clinical pathology conferences that include input from infectious diseases, pulmonology, and other specialties. To train our first model, which was specifically designed to identify CRKP strains, we carefully reviewed specimen data. A total of 297 specimens lacking MALDI-TOF MS data were excluded from the analysis. Subsequently, the refined dataset comprised 2803 cases of carbapenem-susceptible *Klebsiella pneumoniae* (CSKP) and 1207 cases identified as CRKP based on our defined criteria. This model training was integral to our objective of effectively distinguishing between the CRKP and CSKP strains.

Specimen Selection for the CoRKP Training Model

The second model focused on identifying CoRKP. For this analysis, strains classified under the colistin "Intermediate" resistance category (n = 2361) and 151 strains lacking MALDI-TOF MS data were excluded. Consequently, the dataset for this model included 1379 colistin-susceptible *Klebsiella pneumoniae* (CoSKP) and 416 CoRKP.

Data Preprocessing and Feature Extraction

Data Retrieval

Our study utilized Python version 3.9.18. The MSExperiment and MzMLFile classes from the pyopenms library were used to extract mass-to-charge (m/z) ratio segments and intensity values from MALDI-TOF MS results, converting the data into floating-point numbers with Pandas DataFrame. These data were then merged with clinical susceptibility outcomes, where CRKP/CoRKP was labeled as "1" and CSKP/CoSKP as "0".

Balancing the Dataset

Because the number of resistance strains was relatively less compared to the susceptible strain, to effectively address the data imbalance in our study, we implemented a strategic resampling approach using the resampling function from sklearn.utils. This involves downsampling susceptible classes by randomly selecting a subset of samples and upsampling resistant classes by replicating existing samples. These adjustments ensured a balanced dataset that was representative of different cases to prevent model bias and enhance generalizability. Through resampling, the tendency of the predictive model was mitigated to be skewed towards more commonly susceptible cases. This approach preserved crucial data features like protein m/z ratio segments and intensity values for each sample. Retaining such key features from a diverse dataset is crucial for unbiased model training and accurate feature extraction, ultimately ensuring the integrity and robustness of our training process.

Feature Engineering

The m/z ranges were segmented into 1-unit intervals, incorporating a ± 2 error range to categorize similar values. For each segment, peak intensity values were extracted for both antibiotic-resistant and non-resistant strains. To normalize the data, the values were subjected to log transformation. Comparative analysis was performed to distinguish the segments showing the most significant differences in peak values between the two strain types. In instances of overlap between different m/z ratio segments, the segments were merged to ensure a seamless and coherent feature set for analysis. Next, we conducted a feature importance analysis on all sequence segments. Next, we conducted a feature importance analysis on all sequence segments, ranking the features in descending order of importance. During this process, any segment with an importance value lower than 0.01 is considered background noise and is excluded as a feature. This preliminary screening ensured that only the most significant features were used to train the models.

Machine Learning Model Development and Training

In the development and training of our machine learning models, a diverse range of algorithms was employed to explore their predictive capabilities in the context of resistance. This ensemble includes Logistic Regression (LR), Linear

Discriminant Analysis (LDA), a Random Forest Classifier (RFC), a Gradient Boosting Classifier (GBC), an AdaBoost Classifier (ABC), Extreme Gradient Boosting (XGBoost), a Light Gradient Boosting Machine (LGBM), and a Support Vector Machine (SVM).[18] LR is an efficient binary classification algorithm that models the probability of a binary outcome, providing clear and interpretable results with probabilistic predictions. LDA is an efficient classification algorithm for dimensionality reduction that is optimized to maximize class separability and improve classification accuracy. The RFC employs an ensemble of decision trees to improve prediction accuracy and reduce overfitting by combining their individual outputs. GBC is a potent machine learning method that enhances accuracy by sequentially combining multiple weak models to correct their errors, though it demands substantial computational resources. ABC improves classification by iteratively training weak classifiers, adjusting instance weights to address errors, and combining their weighted predictions for enhanced accuracy. XGBoost is a fast and efficient gradient-boosting tool that handles large datasets well, offering features like regularization, parallel processing, and tree pruning for improved accuracy. LGBM is a fast and efficient gradient-boosting framework that handles large datasets with low memory usage and high accuracy, featuring leaf-wise tree growth and parallel learning. SVM is a strong classification tool that works well with complex data. It separates classes clearly and can handle both straightforward and complex relationships using special functions called kernels. These models were selected to encompass a wide array of predictive approaches, each offering unique strengths in classification tasks, to gain a comprehensive perspective on both the individual and combined model efficiencies in accurately prediction. To ensure our models achieve high performance in real-world clinical applications and enhance the predictive accuracy and reliability of our AI-CDSS, we fine-tuned them using sklearn.model_selection library, specifically applying GridSearchCV with 5-fold cross-validation to optimize the area under the curve (AUC). Supplementary Table 1 provides detailed results of the optimal parameters. Samples collected from January to September 2022 were used for training, while those collected from October to December 2022 were used for validation.

Assessing the predictive accuracy of these models is a critical part of our methodology. To evaluate their performance, we employed several metrics, such as the ROC curves and AUC, which are standard measures for visualizing and quantifying the performance of classification models. Additionally, the sensitivity and specificity were calculated to measure the true positive and negative rates, respectively. Positive predictive value (PPV) and negative predictive value (NPV) were used to indicate the likelihood of correct predictions. Finally, the F1-score, which is the harmonic mean of precision and recall, was computed to provide a balanced view of the predictive accuracy of the models. These comprehensive evaluations ensure the reliability and robustness of our findings, making a significant contribution to the field of antibiotic resistance prediction.

AI-CDSS Deployment

To enhance the precision of diagnoses and aid clinical decision-making, we developed a web-based interface specifically designed for medical professionals. This interface leverages sophisticated machine learning algorithms to quickly evaluate the risk of infection with CRKP and CoRKP. A detailed outline of our methodological process, from the selection of specimens to the deployment of the AI-CDSS, is provided in Figure 1.

Results

Mass Spectrometry Analysis of Antibiotic Resistance

Mass spectrometry analysis revealed distinct intensity profiles that can differentiate carbapenem-resistant from carbapenem-susceptible strains and colistin-resistant from colistin-susceptible strains of KP. Figure 2A shows that CRKP strains exhibit high-intensity peaks at m/z segments around 2764, 3150, 4605, 5822, and 6152, which are significantly elevated compared to carbapenem-susceptible strains. Similarly, Figure 2B demonstrates that colistin-resistant strains have pronounced peaks at m/z ratio segments of 4154, 4341, 4571, 5252, 6596, 7158, 8993, and 9252, clearly differentiating them from colistin-susceptible strains. These specific m/z segments with marked intensity variations underscore the spectral differences indicative of resistance in KP, emphasizing the ability of mass spectrometry to identify resistant phenotypes.

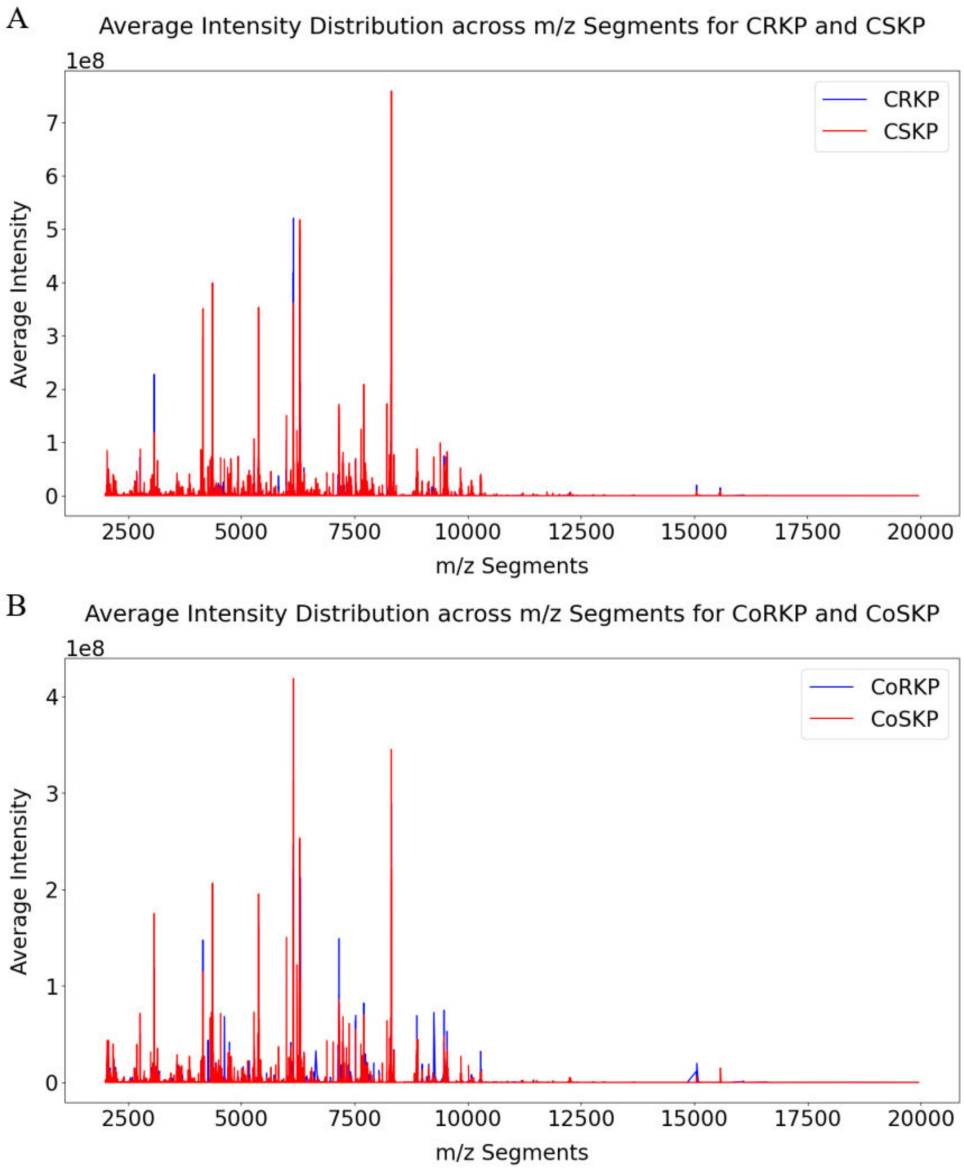

Figure 2 Comparative analysis of average intensity distribution in m/z ratio segments for different *Klebsiella pneumoniae* strains. This figure illustrates the variations in average intensity across mass-to-charge (m/z) ratio segments. (**A**) shows the mass spectrum for carbapenem-resistant *Klebsiella pneumoniae* (CRKP, depicted in blue) compared to carbapenem-susceptible *Klebsiella pneumoniae* (CSKP, depicted in red), highlighting their distinct spectral profiles. (**B**) depicts the mass spectrum for colistin-resistant *Klebsiella pneumoniae* (CoRKP, depicted in blue) versus colistin-susceptible *Klebsiella pneumoniae* (CoSKP, depicted in red), showcasing the spectral differences.

Mass Spectrometry Profiles and Feature Importance

Figure 3A highlights the m/z ratio segments from 2000 to 5500 and 7500 to 10000 as being particularly significant in feature importance, as indicated by the more intense coloration within the spectrum, with a pronounced peak of importance near an m/z ratio segment of 2762. Likewise, Figure 3B shows elevated importance values in the m/z ratio ranges of 2500–5000 and 7000–10000, with especially strong peaks around m/z ratios of 2762 and 4605. These highlighted segments indicate critical regions in the spectrum for the detection of colistin resistance. Figure 4 displays the mean peak intensity of the top 10 distinguishing features for carbapenem-resistant versus susceptible *K. pneumoniae* (Figure 4A), and colistin-resistant versus susceptible strains (Figure 4B). The bar charts in both figures exhibit the differential expression of m/z ratio segments, providing a visual metric of resistance markers.

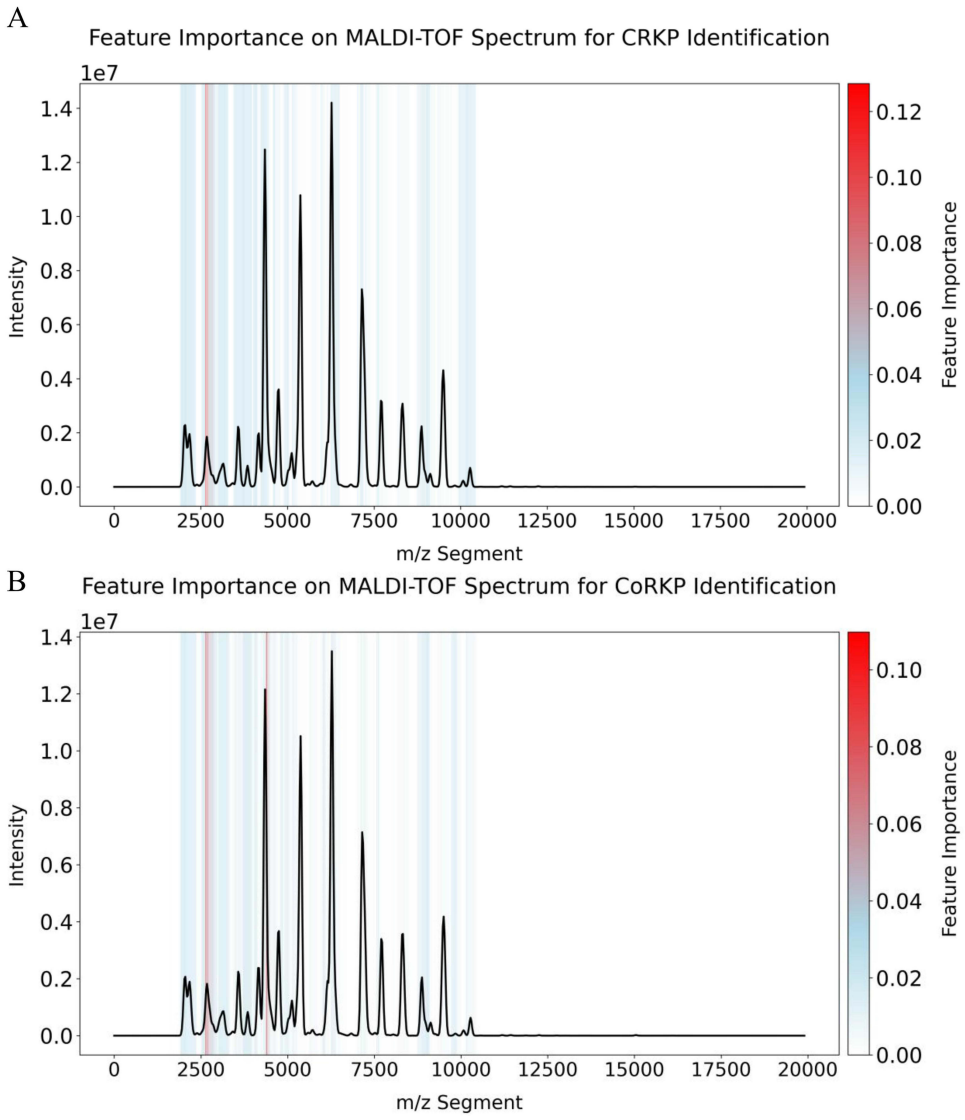

Figure 3 MALDI-TOF spectrum with feature importance for *Klebsiella pneumoniae*. This figure illustrates the feature importance on the MALDI-TOF spectrum for identifying carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and colistin-resistant *Klebsiella pneumoniae* (CoRKP). The color gradient from blue to red indicates increasing feature importance, emphasizing critical regions in the spectrum for detecting resistance. (**A**) shows the intensity distribution across the m/z ratio segments for CRKP. (**B**) displays the intensity distribution for CoRKP.

Model Performance Metrics, Validation Results, and ROC Curve Analyses

Sample collected from October to December 2022 were used for validation, and the performance metrics for the machine learning models used to classify antibiotic-resistant KP strains are detailed in Tables 1 and 2. The RFC demonstrated the highest performance in CRKP models, achieving a validation AUC of 0.96, accuracy of 0.89, sensitivity of 0.88, specificity of 0.90, PPV of 0.89, NPV of 0.89, and an F1-score of 0.88 (Table 1 and Figure 5A). For colistin models, RF led with a validation AUC of 0.98, accuracy of 0.92, sensitivity of 0.94, specificity of 0.91, PPV of 0.91, NPV of 0.93, and an F1-score of 0.93 (Table 2 and Figure 5B).

The XGB and GBC also showed strong performance, with validation AUCs of 0.95 for CRKP and 0.96 for CoRKP. Specifically, XGB achieved an accuracy of 0.87, sensitivity of 0.89, specificity of 0.86, PPV of 0.85, NPV of 0.90, and an F1-score of 0.87 for CRKP (Table 1). For CoRKP, XGB achieved an accuracy of 0.93, sensitivity of 0.95, specificity of 0.90, PPV of 0.91, NPV of 0.95, and an F1-score of 0.93 (Table 2).

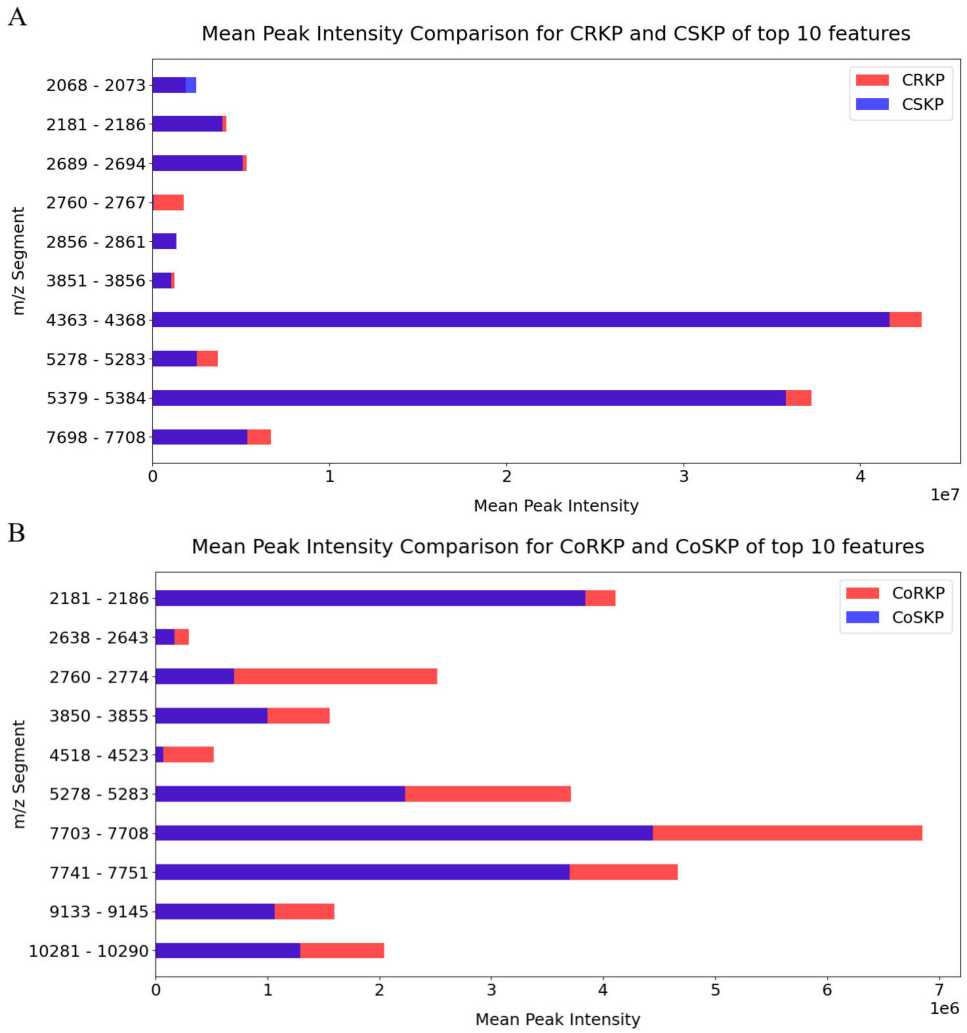

Figure 4 Mean peak intensity comparison for *Klebsiella pneumoniae* strains. The bar charts illustrate the differential expression of these m/z segments, providing a visual metric of resistance markers, with carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and colistin-resistant *Klebsiella pneumoniae* (CoRKP) shown in red and carbapenem-susceptible *Klebsiella pneumoniae* (CSKP) and colistin-susceptible *Klebsiella pneumoniae* (CoSKP) shown in blue. (**A**) compares the mean peak intensity for the top 10 distinguishing m/z ratio segments between CRKP and CSKP, which include 2068–2073, 2181–2186, 2689–2694, 2760–2767, 2856–2861, 3851–3856, 4363–4368, 5278–5283, 5379–5384, and 7698–7708. (**B**) compares the mean peak intensity for the top 10 distinguishing m/z ratio segments between CoRKP and CoSKP, which include 2181–2186, 2638–2643, 2760–2774, 3850–3855, 4518–4523, 5278–5283, 7703–7708, 7741–7751, 9133–9145, and 10281–10,290.

GB showed a validation AUC of 0.95 for CRKP, with an accuracy of 0.89, sensitivity of 0.90, specificity of 0.88, PPV of 0.87, NPV of 0.90, and an F1-score of 0.88 (Table 1). For CoRKP, GB achieved a validation AUC of 0.96, with an accuracy of 0.91, sensitivity of 0.93, specificity of 0.90, PPV of 0.90, NPV of 0.93, and an F1-score of 0.92 (Table 2).

Table 1 Overview of Machine Learning Models' Performance for CRKP and CSKP

Model	Training AUC	Testing AUC	Accuracy	Sensitivity	Specificity	PPV	NPV	F1-score
Random Forest Classifier	1.00	0.96	0.89	0.88	0.90	0.89	0.89	0.88
XGBoost	1.00	0.95	0.87	0.89	0.86	0.85	0.90	0.87
GradientBoosting Classifier	1.00	0.95	0.89	0.90	0.88	0.87	0.90	0.88
LGBM	1.00	0.94	0.88	0.90	0.87	0.87	0.90	0.88
AdaBoost Classifier	1.00	0.91	0.84	0.89	0.79	0.79	0.89	0.84
Logistic Regression	0.80	0.74	0.67	0.75	0.61	0.63	0.72	0.69
Linear Discriminant Analysis	0.75	0.70	0.64	0.67	0.61	0.61	0.67	0.64
Support Vector Machine	0.72	0.65	0.59	0.65	0.54	0.56	0.63	0.60

Abbreviations: AUC, area under the curve; CRKP, carbapenem-resistant *Klebsiella pneumonia*; CSKP, carbapenem-susceptible *Klebsiella pneumonia*, LGBM, light gradient boosting machine; NPV, negative predictive value; PPV, positive predictive value; XGBoost, eXtreme gradient boosting.

Table 2 Overview of Machine Learning Models' Performance for CoRKP and CoSKP

Model	Training AUC	Testing AUC	Accuracy	Sensitivity	Specificity	PPV	NPV	F1-score
Random Forest Classifier	1.00	0.98	0.92	0.94	0.91	0.91	0.93	0.93
XGBoost	1.00	0.96	0.93	0.95	0.90	0.91	0.95	0.93
GradientBoosting Classifier	1.00	0.96	0.91	0.93	0.90	0.90	0.93	0.92
LGBM	1.00	0.96	0.91	0.93	0.89	0.90	0.93	0.92
AdaBoost Classifier	1.00	0.95	0.91	0.94	0.89	0.90	0.93	0.92
Logistic Regression	0.90	0.79	0.73	0.68	0.78	0.76	0.71	0.72
Linear Discriminant Analysis	0.87	0.75	0.68	0.60	0.76	0.72	0.65	0.65
Support Vector Machine	0.79	0.75	0.67	0.46	0.87	0.79	0.61	0.58

Abbreviations: AUC, area under the curve; CoRKP, colistin-resistant *Klebsiella pneumonia*; CoSKP, colistin-susceptible *Klebsiella pneumonia*; LGBM, light gradient boosting machine; NPV, negative predictive value; PPV, positive predictive value; XGBoost, eXtreme gradient boosting.

The ROC curves in Figures 5A and 5B illustrate the strong predictive performance of these models. RFC, XGB, and GBC demonstrated a steep ascent towards the top-left corner of the ROC space, indicating high true-positive rates and low false-positive rates. LR, LDA, and SVM models, although exhibiting lower AUC values, still contributed valuable insights to the ensemble approach, providing a comprehensive assessment of antibiotic resistance in KP.

Overall, the ensemble of models demonstrated a range of AUC values, with RFC consistently exhibiting top-tier performance. By incorporating multiple metrics such as accuracy, sensitivity, specificity, PPV, NPV, and F1-score, we ensured a robust evaluation of model performance, highlighting the strengths of each algorithm in predicting antibiotic resistance accurately and reliably.

Integration of AI-CDSS in Clinical Practice

The Introduction of an AI-CDSS for real-time assessment of *Klebsiella pneumoniae*'s resistance to carbapenems and colistin represents a crucial advancement in the field of clinical diagnostics. By utilizing machine learning algorithms to analyze mass spectrometry data, this system can predict resistance levels, providing healthcare professionals with probability scores. These scores assist in making quick and informed treatment decisions, streamlining the clinical workflow significantly. Our AI-CDSS can determine resistance in approximately three days, surpassing the traditional four-day period for standard AST. Compared to traditional diagnostic methods, this AI integration allows for earlier intervention, which could lead to improved outcomes for patients by ensuring they receive the most effective treatment promptly.

Discussion

Principal Results

By using advanced MALDI-TOF MS technology and various machine learning algorithms, models were trained to predict carbapenem and colistin resistance, aiming to address the global health issues caused by CRKP and CoRKP. AI-CDSS integrated with these machine learning algorithms could accelerate antibiotic resistance detection and treatment, providing healthcare workers a reliable tool for fast and accurate antibiotic use. This approach could lead to better patient care and more efficient treatment by quickly identifying resistant strains.

Spectral Analysis and Feature Importance

Spectral analysis provided unique insights into the resistance mechanisms of CRKP and CoRKP, demonstrating specific spectral differences between antibiotic-resistant and antibiotic-susceptible strains. Notably, the m/z ratio segments 2764, 3150, 4605, 5822, and 6152 for CRKP and 4154, 4341, 4571, 5252, 6596, 7158, 8993, and 9252 for CoRKP were significantly elevated compared to their susceptible counterparts. Figure 3A highlights the m/z ratio segments from 2000 to 5500 and 7500 to 10000 as particularly significant in feature importance, with pronounced peaks near 2762. Similarly, Figure 3B shows elevated importance values in the m/z ratio ranges of 2500–5000 and 7000–10000, with strong peaks around 2762 and 4605. Figure 4 displays the mean peak intensity of the top 10 distinguishing features for carbapenem-

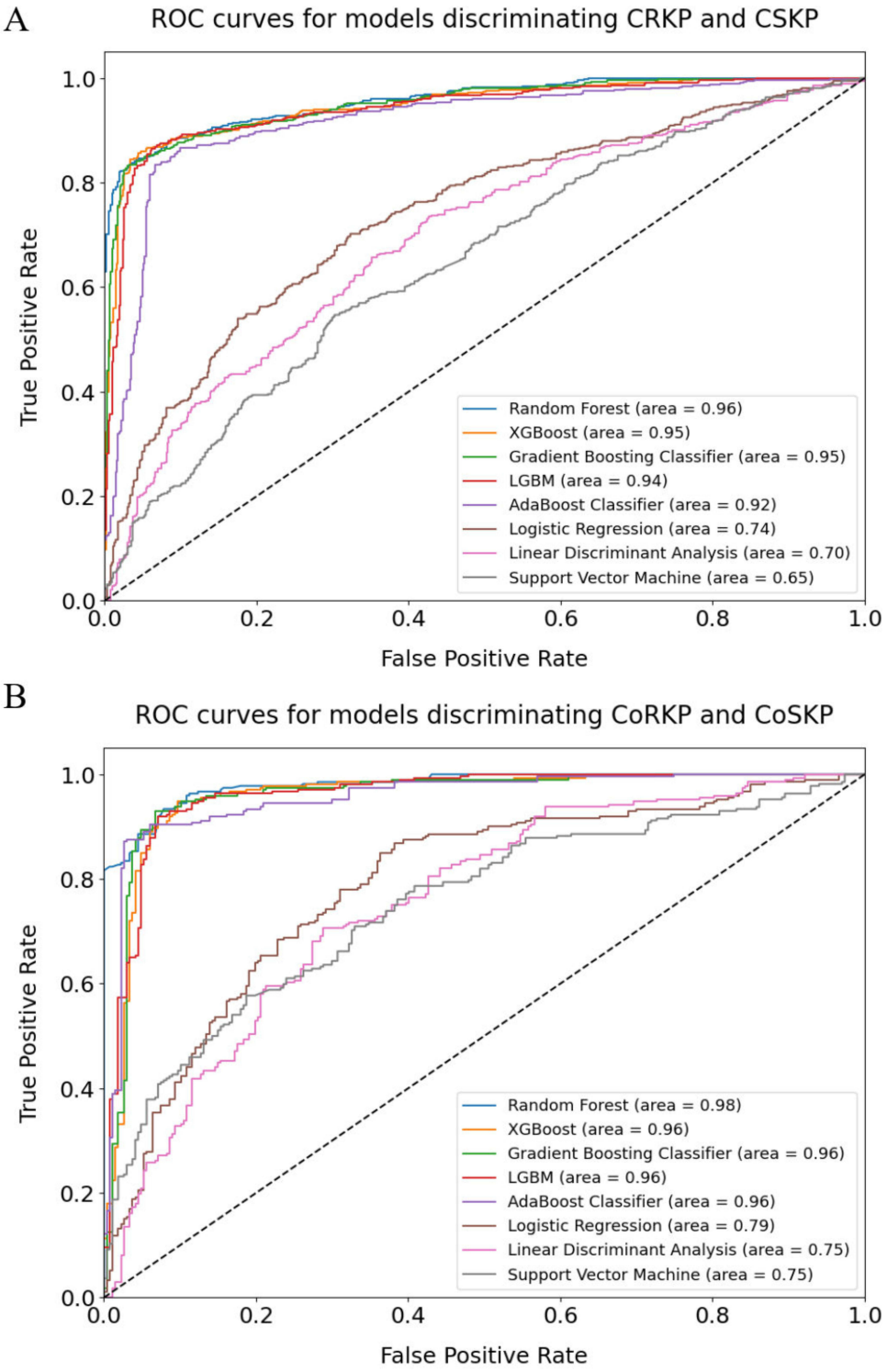

Figure 5 Receiver operating characteristic (ROC) curves evaluating the performance of various machine learning classifiers. (**A**) illustrates the true-positive rate against the false-positive rate for detecting carbapenem-resistant *Klebsiella pneumoniae* (CRKP), with the area under the curve (AUC) indicating each classifier's discriminative power. (**B**) shows the ROC curve for detecting colistin-resistant *Klebsiella pneumoniae* (CoRKP), highlighting the classifiers' effectiveness and AUC values reflecting their accuracy in distinguishing resistant strains.

resistant versus susceptible KP and colistin-resistant versus susceptible strains. These findings demonstrate the efficacy of MALDI-TOF MS in distinguishing between resistant and susceptible strains and highlight the critical role of machine learning in interpreting complex biological data. This synergy between advanced technologies offers a new approach to

combating antibiotic resistance, supporting the goal of integrating precise MALDI-TOF MS data with machine learning to identify antibiotic resistance and optimize treatment decisions more efficiently.

Advancing Diagnostics

The escalating threat of antibiotic resistance, exemplified by CRKP strains, underscores the urgent need for innovative diagnostic methods.[3,5,6] Traditional diagnostic tools often fall short of promptly detecting these resistant bacteria, leading to delays in appropriate treatment and control measures.[19,20] Studies are increasingly using machine learning to predict antimicrobial resistance by processing patient demographics, medical history, laboratory results, and electronic health record data, including unstructured physician notes.[21] This trend involves integrating machine learning models into these records to analyze real-time data, predict antibiotic susceptibility, and provide tailored treatment recommendations. In contrast, our study utilized solely the MALDI-TOF MS profile, further simplifying the data collection process by reducing the time required for tasks such as history taking and other laboratory testing. Our research establishes the most extensive collection of isolates to date for constructing a machine-learning model aimed at identifying CRKP and associated colistin resistance. The methodological innovation of this study, through the analysis of over 8000 KP cultures, demonstrates the feasibility and accuracy of using advanced technologies to predict resistance patterns. This approach not only enhances the precision of diagnostics but also significantly reduces the time required to identify resistant strains, facilitating timely and targeted therapeutic interventions.

The application of machine learning models, such as the RFC, GBC, and XGBoost, in predicting antibiotic resistance marks a significant leap forward. The models' high AUC scores indicated their effectiveness in distinguishing between CRKP and CSKP strains, as well as colistin-resistant and colistin-susceptible strains. This predictive accuracy is crucial for antibiotic stewardship as it enables clinicians to make informed decisions regarding antibiotic usage, thereby mitigating the spread of resistance. By enabling quicker decision-making, the implementation of machine learning models supports crucial antibiotic stewardship efforts, ensuring that treatments are both targeted and appropriate.[22]

In our current AI-CDSS, CRKP is categorized as either "Resistant" or "Intermediate" for IPM or DOR in AST to meet clinical needs, a definition that was established through consensus at our multidisciplinary meetings and aligns with the study by Cienfuegos-Gallet et al.[23] Intermediate resistance suggests bacteria have evolved mechanisms that reduce antibiotic effectiveness, including decreased permeability, efflux pumps, or mutations in target sites.[24] This indicates uncertain therapeutic outcomes, which may necessitate higher dosing, though efficacy depends on the concentration at the infection site and the patient's tolerance to potential side effects from increased dosage.[25] Our hospital consensus recommends avoiding carbapenems when sensitivity is reported as "Intermediate", preferring alternative antibiotics to enhance treatment efficacy and prevent resistance development. This approach reflects our commitment to personalized patient care and the prudent use of antibiotics to combat antimicrobial resistance. The AI-CDSS was developed to streamline the clinical consensus and treatment decision-making process more efficiently. Each facility has the potential to design its own AI-CDSS to tailor to their specific needs.

Impact and Future Directions

In addition to the advancements detailed above, the integration of an AI-CDSS stands as a groundbreaking enhancement to our diagnostic capabilities. AI-CDSS, leveraging the predictive power of machine learning models, offers clinicians real-time, data-driven insights, significantly improving the decision-making process in the treatment of infections caused by carbapenem-resistant and colistin-resistant KP. This system can rapidly interpret complex diagnostic data, recommend personalized treatment options, and predict potential resistance patterns, thereby not only streamlining the diagnostic workflow but also facilitating a more targeted approach to antibiotic therapy. The introduction of AI-CDSS into our methodology underscores our commitment to harnessing cutting-edge technology to combat antibiotic resistance, representing a vital step forward in the optimization of clinical outcomes and the advancement of antibiotic stewardship.

Future investigations should focus on broadening the scope of our dataset to incorporate specimens from a wide array of geographical locations and clinical environments. This expansion will enhance the model's generalizability and robustness. Concurrently, the implementation of our AI-CDSS across a diverse range of healthcare institutions will provide invaluable insights into its performance in varied real-world scenarios. These

strategic initiatives are poised to catalyze significant advancements in the domain of antimicrobial resistance detection and management. By refining our approach, we aim to develop a more sophisticated and holistic diagnostic instrument capable of addressing the complex challenges posed by antibiotic-resistant pathogens in diverse healthcare settings.

Limitations

While our study demonstrates significant advances in the detection and management of antibiotic-resistant pathogens using AI-CDSS, several Limitations should be acknowledged. The use of data from a single hospital and four secondary hospitals may limit the generalizability of our findings across different geographical areas and healthcare settings, which might vary between institutions. Additionally, the study's accuracy is contingent upon the quality of the underlying data, including MALDI-TOF MS profiles and antibiotic susceptibility tests. There is also an inherent uncertainty in machine learning models, which could affect their predictive accuracy, particularly for rare or novel resistance mechanisms. The model's performance may be less reliable in detecting new or uncommon resistance patterns that were not well represented in our training data. Moreover, our focus on carbapenem and colistin resistance addresses only a segment of the broader antibiotic resistance issue, necessitating the expansion of AI-CDSS to encompass a wider range of pathogens and resistance mechanisms. This selective focus might overlook other significant resistance types that are critical in different clinical settings. The successful integration of AI-CDSS into clinical workflows also presents logistical and acceptance challenges, highlighting the need for future research to enhance data collection methods, extend the system's scope, and address the practical challenges of implementing such technology in clinical settings.

Conclusion

This study demonstrates the potential of AI-CDSS combining MALDI-TOF MS technology with machine learning for advancing diagnostic capabilities against antibiotic-resistant bacteria. Our findings pave the way for better diagnostics and treatments. The proposed AI-CDSS could significantly reduce the identification time of resistance strain from a day to minutes, allowing for faster, more targeted interventions. This advancement could potentially revolutionize how we manage infections and use antibiotics, addressing the growing threat of antimicrobial resistance.

Abbreviations

ABC, AdaBoost classifier; AUC, area under the curve; AI-CDSS, artificial intelligence-clinical decision support system; CLSI, Clinical and Laboratory Standards Institute; CoRKP, colistin-resistant *Klebsiella pneumoniae*; CoSKP, colistin-susceptible *Klebsiella pneumoniae*; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; CSKP, carbapenem-susceptible *Klebsiella pneumoniae*; DOR, doripenem; GBC, gradient boosting classifier; IPM, imipenem; LDA, linear discriminant analysis; LGBM, light gradient boosting machine; LR, logistic regression; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; m/z, mass-to-charge ratio; NPV, negative predictive value; PPV, positive predictive value; RFC, random forest classifier; ROC, receiver operating characteristic; SVM, support vector machine; XGBoost, eXtreme gradient boosting.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author, upon reasonable request.

Ethics Approval and Informed Consent

This study was approved by the Tri-Service General Hospital Institutional Review Board (TSGHIRB No. C202305073) and conducted in accordance with the ethical standards of the Declaration of Helsinki. Due to the retrospective design of the study, the need for patient consent was waived. This waiver was approved by the TSGHIRB because the research involves no more than minimal risk to the subjects and involves the use of existing data, records, and specimens in such

a manner that subjects cannot be identified directly or through identifiers linked to the subjects. All patient data were handled confidentially to ensure privacy and compliance with data protection regulations.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed; 2017. Available from: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. Accessed April 16, 2024.
2. Bulman ZP, Krapp F, Pincus NB, et al. Genomic features associated with the degree of phenotypic resistance to carbapenems in carbapenem-resistant Klebsiella pneumoniae. *mSystems*. 2021;6(5):e0019421. doi:10.1128/mSystems.00194-21
3. Hu Y, Liu C, Shen Z, et al. Prevalence, risk factors and molecular epidemiology of carbapenem-resistant Klebsiella pneumoniae in patients from Zhejiang, China, 2008–2018. *Emerg Microbes Infect*. 2020;9(1):1771–1779. doi:10.1080/22221751.2020.1799721
4. Lin XC, Li CL, Zhang SY, Yang XF, Jiang M. The global and regional prevalence of hospital-acquired carbapenem-resistant Klebsiella pneumoniae infection: a systematic review and meta-analysis. *Open Forum Infect Dis*. 2024;11:ofad649. doi:10.1093/ofid/ofad649
5. Nation RL, Li J, Cars O, et al. Framework for optimisation of the clinical use of colistin and polymyxin B: the Prato polymyxin consensus. *Lancet Infect Dis*. 2015;15(2):225–234. doi:10.1016/S1473-3099(14)70850-3
6. Zhang X, Guo F, Shao H, Zheng X. Clinical translation of polymyxin-based combination therapy: facts, challenges and future opportunities. *J Infect*. 2017;74(2):118–130. doi:10.1016/j.jinf.2016.11.015
7. Agyeman AA, Bergen PJ, Rao GG, Nation RL, Landersdorfer CB. A systematic review and meta-analysis of treatment outcomes following antibiotic therapy among patients with carbapenem-resistant Klebsiella pneumoniae infections. *Int J Antimicrob Agents*. 2020;55(1):105833. doi:10.1016/j.ijantimicag.2019.10.014
8. Xu L, Sun X, Ma X. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant Klebsiella pneumoniae. *Ann Clin Microbiol Antimicrob*. 2017;16(1):18. doi:10.1186/s12941-017-0191-3
9. Huang W, Qiao F, Zhang Y, et al. In-hospital medical costs of infections caused by carbapenem-resistant Klebsiella pneumoniae. *Clin Infect Dis*. 2018;67(suppl_2):S225–S230. doi:10.1093/cid/ciy642
10. Garcia E, Diep JK, Sharma R, Rao GG. Model-based learn and confirm: designing effective treatment regimens against multidrug resistant gram-negative pathogens. *Int J Antimicrob Agents*. 2024;63(4):107100. doi:10.1016/j.ijantimicag.2024.107100
11. Wang Y, Cong S, Zhang Q, Li R, Wang K. iTRAQ-Based proteomics reveals potential anti-virulence targets for ESBL-producing Klebsiella pneumoniae. *Infect Drug Resist*. 2020;13:2891–2899. doi:10.2147/IDR.S259894
12. Weng Y, Wang T, Huang B, et al. Multicenter Study of Colistin Heteroresistance in Carbapenem-Resistant Klebsiella pneumoniae Strains in China. *Microbiol Spectr*. 2023;11(4):e0221822. doi:10.1128/spectrum.02218-22
13. Zhou C, Wu Q, He L, et al. Clinical and molecular characteristics of carbapenem-resistant hypervirulent Klebsiella pneumoniae isolates in a tertiary hospital in Shanghai, China. *Infect Drug Resist*. 2021;14:2697–2706. doi:10.2147/IDR.S321704
14. Weis CV, Jutzeler CR, Borgwardt K. Machine learning for microbial identification and antimicrobial susceptibility testing on MALDI-TOF mass spectra: a systematic review. *Clin Microbiol Infect*. 2020;26(10):1310–1317. doi:10.1016/j.cmi.2020.03.014
15. Theodosiou AA, Read RC. Artificial intelligence, machine learning and deep learning: potential resources for the infection clinician. *J Infect*. 2023;87(4):287–294. doi:10.1016/j.jinf.2023.07.006
16. Jang KS, Kim YH. Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. *J Microbiol*. 2018;56(4):209–216. doi:10.1007/s12275-018-7457-0
17. Humphries RM, Abbott AN, Hindler JA, Kraft CS. Understanding and addressing CLSI breakpoint revisions: a primer for clinical laboratories. *J Clin Microbiol*. 2019;57(6):e00203–19. doi:10.1128/JCM.00203-19
18. Khan AA, Chaudhari O, Chandra R. A review of ensemble learning and data augmentation models for class imbalanced problems: combination, implementation and evaluation. *Expert Syst Appl*. 2024;3:244.

19. Band VI, Satola SW, Burd EM, Farley MM, Jacob JT, Weiss DS. Carbapenem-resistant Klebsiella pneumoniae exhibiting clinically undetected colistin heteroresistance leads to treatment failure in a murine model of infection. *mBio*. 2018;9(2):e02448–17. doi:10.1128/mBio.02448-17
20. Bleumin D, Cohen MJ, Moranne O, et al. Carbapenem-resistant Klebsiella pneumoniae is associated with poor outcome in hemodialysis patients. *J Infect*. 2012;65(4):318–325. doi:10.1016/j.jinf.2012.06.005
21. Sakagianni A, Koufopoulou C, Feretzakis G, et al. Using machine learning to predict antimicrobial resistance-A literature review. *Antibiotics*. 2023;12(3):452. doi:10.3390/antibiotics12030452
22. Feretzakis G, Sakagianni A, Loupelis E, et al. Using machine learning to predict antimicrobial resistance of Acinetobacter baumannii, Klebsiella pneumoniae and pseudomonas aeruginosa strains. *Stud Health Technol Inform*. 2021;281:43–47. doi:10.3233/SHTI210117
23. Cienfuegos-Gallet AV, Zhou Y, Ai W, Kreiswirth BN, Yu F, Chen L. Multicenter genomic analysis of carbapenem-resistant Klebsiella pneumoniae from bacteremia in China. *Microbiol Spectr*. 2022;10(2):e0229021. doi:10.1128/spectrum.02290-21
24. Cloete TE. Resistance mechanisms of bacteria to antimicrobial compounds. *Int Biodeterior Biodegrad*. 2003;51(4):277–282. doi:10.1016/S0964-8305(03)00042-8
25. Hombach M, Bottger EC, Roos M. The critical influence of the intermediate category on interpretation errors in revised EUCAST and CLSI antimicrobial susceptibility testing guidelines. *Clin Microbiol Infect*. 2013;19(2):E59–71. doi:10.1111/1469-0691.12090