

# A Machine Learning-Based Diagnostic Utility for Predicting Ertapenem Resistance in *Klebsiella pneumoniae* Using MALDI-TOF MS Spectra

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**Abstract**—*Klebsiella pneumoniae* is a gram-negative bacterium that causes over 600,000 deaths annually worldwide. Carbapenems, a powerful class of  $\beta$ -lactam antibiotics, are used to treat *Klebsiella pneumoniae*, as a last-resort when other antibiotics fail. However, when bacteria become resistant to carbapenems, treatment options become very limited. Ertapenem, a commonly used member of the carbapenems family, is widely applied in clinical settings, making early detection of ertapenem resistance crucial for preventing the spread of resistant strains and improving patient outcomes. Traditional methods such as Polymerase Chain Reaction, Whole-Genome Sequencing, and Gene Expression Analysis remain limited because they require long processing times, rely on complex and high-cost equipment, and may not reliably detect new/uncommon resistance mechanisms. Newer approaches such as MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) offer rapid bacterial identification, but determining whether isolates are resistant or susceptible to ertapenem still requires additional time-consuming manual processes. In this study, we integrated several machine learning (ML) algorithms: Logistic Regression, K-Nearest Neighbors (KNN), Multi-Layer Perceptron (MLP), and Random Forest, with MALDI-TOF MS spectra to predict ertapenem resistance with high accuracy. The results were promising: Logistic Regression, MLP, and Random Forest achieved testing accuracies of 86%, 84%, and 78%. This approach has strong potential to improve the diagnostic capability of MALDI-TOF MS by enabling rapid and accurate detection of *Klebsiella pneumoniae* resistance and supporting timely, targeted decision-making. Unlike previous research, which has been more generic in scope, this study is specifically focused on ertapenem (an easy-to-administer, cost-effective carbapenem) and employs lightweight ML models that can run on standard laboratory computers, making the method more suitable for real-world clinical applications.

## I. INTRODUCTION

*Klebsiella pneumoniae* is a major cause of hospitalized infections and becomes especially dangerous when strains are resistant to carbapenem antibiotics. It causes over 600,000 deaths annually worldwide and accounts for nearly one-third of all gram-negative bacterial infections [1]. Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has emerged as a major global health threat, causing severe infections that are often associated with high rates of morbidity and mortality. These infections are difficult to treat and can lead to serious complications, with mortality rates for pneumonia caused by *Klebsiella pneumoniae* reaching 50% [2]. In the United States, as of 2023, *Klebsiella pneumoniae* causes about 3–8% of hospital-acquired bacterial infections [3]. These organisms commonly live in the gastrointestinal tract [4] and mouth [5], and they spread easily in hospitals through contaminated hands, shared equipment, and frequently touched surfaces [6].

CRKP poses a significant global health threat because only a limited number of antibiotics remain effective against it. Since treatment options are limited and death rates are high, preventing the spread and improving early detection is crucial [7]. Carbapenems are very strong antibiotics that doctors use when other medicines no longer work. They are considered last-resort drugs for serious infections caused by these bacteria. When bacteria become resistant to carbapenems, treatment options become extremely limited, making infections much harder to cure [8]. Among carbapenems, ertapenem serves as an important marker for early detection of resistance, since loss of susceptibility to ertapenem often signals emerging resistance to other carbapenems [9]. Ertapenem is also widely used in clinical practice because it is easy to

administer, simple to deliver, cost-effective, and efficient. Therefore, this research focuses specifically on resistance to this carbapenem.

Traditional microbiological methods, such as Polymerase Chain Reaction (PCR), Whole-Genome Sequencing (WGS), and Gene Expression Analysis, provide detailed molecular information but are limited by long processing times, complex workflows, and the need for specialized, high-cost laboratory infrastructure [10]. In contrast, MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) has emerged as a powerful and efficient alternative, capable of identifying bacterial species within minutes using minimal sample preparation and significantly lower operational cost [11]. MALDI-TOF MS generates a protein-based spectral profile of the organism and matches it against reference databases for rapid identification. Its speed, reproducibility, and ability to handle high sample volumes make it particularly valuable in clinical microbiology laboratories [12].

Identifying *Klebsiella pneumoniae* using MALDI-TOF MS is only the first step. The true clinical benefit materializes when we can rapidly determine whether the isolate is resistant or susceptible to key treatment options such as ertapenem. Determining carbapenem resistance still depends on phenotypic Antimicrobial Susceptibility Testing (AST), such as disk diffusion, broth microdilution, and E-test methods [9]. These tests require bacteria to grow in the presence of antibiotics, which takes many hours or even days. Due to this slow process, treatment decisions are delayed, leading to worse patient outcomes and a greater risk of resistant strains spreading in healthcare settings [13].

In light of these challenges and opportunities, the primary question this research addresses is whether ertapenem resistance in *Klebsiella pneumoniae* can be predicted by integrating MALDI-TOF MS spectra with machine learning models, and which specific model (Logistic Regression, KNN, MLP, and Random Forest) provides the most accurate and reliable performance. This research is motivated by the need for faster resistance determination in clinical settings where slow, culture-based testing delays appropriate treatment. The objective is to provide a practical and accessible diagnostic utility that supports earlier and more accurate detection of ertapenem resistance in *Klebsiella pneumoniae* isolates.

## II. RELATED WORK

Several recent studies have demonstrated the potential of ML and deep learning (DL) approaches in addressing

*Klebsiella pneumoniae* antimicrobial resistance. Peng developed a convolutional neural network (CNN) based model to screen 2,475 compounds from the DrugBank database, achieving an accuracy of 72 to 75% in predicting drug target interactions [14]. In another study, Moat employed a genome-based prediction using XGBoost, SVM, and neural network models on thousands of *E. coli* genomes. SVM and XGBoost achieved approximately 88% accuracy [15]. These early works highlight the potential of supervised learning models for antimicrobial resistance (AMR) prediction using genomic or chemical data but depend heavily on sequence-level information and high computing requirements, which limits real-time use in clinical workflows.

A study by Condorelli used machine learning to predict antibiotic resistance in *Klebsiella pneumoniae* based on genomic data, analyzing the presence or absence of resistance genes across two datasets and training several models to classify strains as resistant or susceptible. Their approach demonstrated high accuracy for multiple antibiotics, showing that genome-based machine learning can support faster and more informed treatment decisions compared to traditional laboratory testing [16]. However, genome-based models require sequencing resources that are costly and time-intensive. This motivated interest in MALDI-TOF MS-based data for faster and lower-cost prediction methods.

Weis developed a machine learning method to predict antimicrobial resistance directly from clinical MALDI-TOF MS spectra, creating a multi-hospital dataset of over 300,000 spectra and achieving accuracy scores of 74% to 84% for major pathogens, including *Klebsiella pneumoniae* [17]. This demonstrated the feasibility of MALDI-TOF based AMR prediction and encouraged further species-focused modeling. While this study showed that MALDI-TOF MS can support general AMR prediction, the approach was not focused on ertapenem resistance in *Klebsiella pneumoniae*. This makes it less useful for antibiotic-specific needs and species-specific predictions.

DL based studies have also advanced MALDI-TOF MS analysis. A 2024 study called MSDeepAMR used DL to classify resistant and susceptible isolates directly from routine MALDI-TOF MS data. Trained on a large public dataset including *E. coli*, *Klebsiella pneumoniae*, and *S. aureus*, the models achieved accuracy above 80% [18]. A comparative study of Logistic Regression, Classification and Regression Trees (CRT), Random Forest, and Artificial Neural Networks (ANN) reported high accuracies in predicting carbapenem-resistant *Klebsiella pneumoniae* [19]. These methods detect nonlinear pat-

terns in the data, but they use complex models that are less interpretable and require more computation for everyday clinical use.

While previous research has shown promising results, most studies have focused broadly on carbapenem resistance, included multiple carbapenems together, or used complex models that are difficult to deploy in everyday clinical workflows. There has not been enough focus specifically on ertapenem resistance as a targeted predictive marker. This study addresses these gaps by building a balanced, species-specific dataset for ertapenem resistance, applying thorough preprocessing of MALDI-TOF MS spectra, and evaluating lightweight machine-learning models. Our work contributes a utility for rapid AMR prediction in *Klebsiella pneumoniae* using ertapenem-specific modeling, making it more aligned with real-world diagnostic needs.

### III. MATERIALS AND METHODS

#### A. Research Question and Approach

The goal of this study was to determine whether ertapenem resistance in *Klebsiella pneumoniae* can be predicted from MALDI-TOF MS spectra using supervised machine learning. Four models were evaluated for this task: Logistic Regression, KNN, MLP, and Random Forest.

The DRIAMS (Database of Resistance Information on Antimicrobials and MALDI-TOF Mass Spectra) dataset was used for this research. DRIAMS contains microbiology data collected from four Swiss medical institutes. In total, the dataset includes more than 300,000 samples from hundreds of bacterial and fungal pathogens, along with their antibiotic susceptibility profiles [20]. MALDI-TOF MS spectra in DRIAMS are stored both in raw text form and in several processed formats. One of these formats, called binned\_6000, divides the mass-to-charge ( $m/z$ ) axis into 3 Dalton intervals which produces 6,000 numerical features per spectrum [20].

#### B. Data Extraction and Preprocessing

The data extraction process began by loading DRIAMS metadata from all four institutes and filtering the dataset to include only *Klebsiella pneumoniae* isolates. Ertapenem labels were identified as susceptible (S), resistant (R), or uncertain. Uncertain entries were reviewed and either corrected or removed. For each remaining isolate, the MALDI-TOF spectrum was extracted and converted into a numerical vector of 6,000 intensity values.

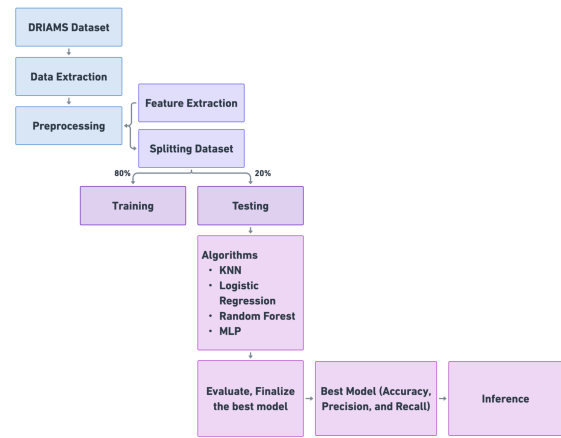


Fig. 1: End-to-end process showing the analysis of MALDI-TOF MS spectra with ML models to identify *Klebsiella pneumoniae* resistance or susceptibility

Several preprocessing operations were applied to reduce noise and improve feature consistency. Rows with missing spectral values were removed to ensure data quality. The spectral matrix ( $X$ ) and ertapenem label vector ( $Y$ ) were defined. A log-based variance stabilization transform was then applied by multiplying each spectral bin by 1000 and computing  $\log(1 + x)$ . This reduced skewed distributions and made the data more suitable for machine learning. After preprocessing, spectra were aligned and binned so that comparable mass spectrum ( $m/z$ ) regions matched across isolates.

#### C. Data Sampling Strategy

This research evaluated several data balancing techniques, including undersampling, Synthetic Minority Over-sampling Technique (SMOTE), Adaptive Synthetic Sampling (ADASYN), and class weighting. Among these approaches, undersampling demonstrated the strongest performance, achieving the highest macro recall and macro F1-score compared to the other methods. Detailed results are presented in the Results section. After this Undersampling exercise, the final working dataset consisted of 397 isolates, with 300 susceptible and 97 resistant samples, compared to the original distribution of 5,069 susceptible, 94 resistant, and 17 uncertain. Recursive Feature Elimination (RFE) using a Random Forest estimator was then applied to identify the most informative bins, reducing the original 6,000 features to 96 features that showed the clearest patterns distinguishing resistant from susceptible isolates.

The prepared dataset was split into training and testing portions using stratified sampling, in which 80% of the data was used for training and 20% for testing.

Stratification ensured that the proportion of susceptible and resistant isolates remained consistent in both sets. The same train–test split was used for all four models to support fair comparison.

#### D. Training, Testing, and Evaluation

Logistic Regression predicted whether an isolate was resistant or susceptible by estimating class probabilities. KNN assigned a class based on the most common label among the closest samples in feature space. The MLP model learned nonlinear relationships within the spectral data and depended on the choice of learning rate, hidden layers, and training epochs. Random Forest built many decision trees and combined their predictions to improve accuracy and stability.

Each model was tuned during the training phase using a grid search combined with K-Fold cross-validation on the training set. For Logistic Regression, the inverse regularization strength (C) was tuned over a log-spaced range (0.01-100) and the maximum number of iterations was set sufficiently high (50-300) to ensure convergence. For the Random Forest model, tree depth (1–7) and the number of estimators (10–100) were systematically explored. The MLP model was trained using learning rates ranging from 0.000001 to 0.05 and epochs from 10 to 100. For KNN, values of k from 1 to 15 were evaluated. Using cross-validation only on the training data helped choose hyperparameters reliably while preventing any information from leaking from the test set.

After tuning, the models were tested on the held-out 20% of the dataset. Performance was evaluated using accuracy, precision, recall, and F1-score, based on four outcomes: a true positive (TP) when the model correctly identifies a resistant isolate, a false positive (FP) when a susceptible isolate is incorrectly predicted as resistant, a true negative (TN) when the model correctly identifies a susceptible isolate, and a false negative (FN) when a resistant isolate is incorrectly predicted as susceptible, defined as:

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

$$\text{Precision} = \frac{TP}{TP + FP}$$

$$\text{Recall} = \frac{TP}{TP + FN}$$

$$\text{F1-score} = 2 \cdot \frac{\text{Precision} \cdot \text{Recall}}{\text{Precision} + \text{Recall}}$$

Accuracy measured the proportion of correct predictions. Precision captured how many isolates predicted as resistant were actually resistant, while recall measured how many true resistant isolates were detected. The F1-score provided a balanced measure that combined both. A normalized confusion matrix and classification report were generated for each model to compare performance across both classes and identify the model that performed best for predicting ertapenem resistance in *Klebsiella pneumoniae*.

## IV. RESULTS

### A. Results at Model Training Stage

Logistic Regression was evaluated using different maximum iteration limits and consistently achieved a training accuracy of 83%, as shown in Figure 2. The flat accuracy trend shows that changing the number of iterations did not impact performance, indicating that the model converged early and that this parameter is not sensitive after convergence. Since this analysis mainly confirms model stability rather than improving performance, overall classification ability was evaluated separately using receiver operating characteristic (ROC) analysis. As shown in Figure 3, the model achieved an Area Under the Curve (AUROC) of 0.83, demonstrating strong performance across classification thresholds. This value is higher than the AUROC of 0.74 reported by Weiss [17] for antimicrobial resistance prediction in *Klebsiella pneumoniae* using a broader machine learning approach. By focusing specifically on ertapenem resistance and using a targeted modeling strategy, this approach shows improved predictive performance compared to the prior baseline. Because the model converged early, further tuning focused on the inverse regularization strength (C), which more directly affects model complexity and generalization.

KNN was evaluated across multiple values for the number of neighbors, with the best performance observed when using 14 neighbors, yielding a training accuracy of 82%. Figure 4 illustrates that the performance of the KNN model depends on the hyperparameters used during tuning. The figure summarizes the training accuracy across different neighbor values and shows that using 14 neighbors (k=14) yields the most stable and accurate results, achieving an accuracy of 82%. This configuration provides more reliable classifications of ertapenem-resistant and susceptible *Klebsiella pneumoniae* compared to other values.

The MLP model achieved a training accuracy of 85.7% with a learning rate of 0.01 and 90 epochs. Figure

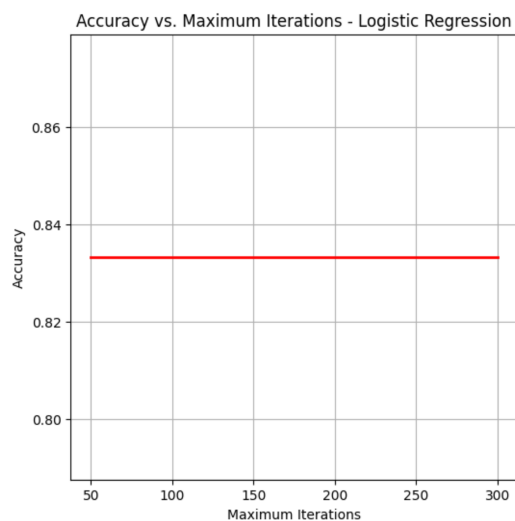


Fig. 2: The accuracy graph for Logistic Regression with maximum iterations vs. accuracy

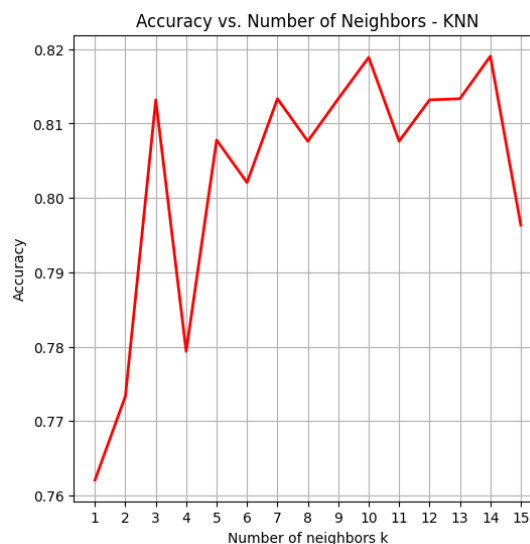


Fig. 4: The accuracy graph for KNN with number of neighbors (k) vs. accuracy

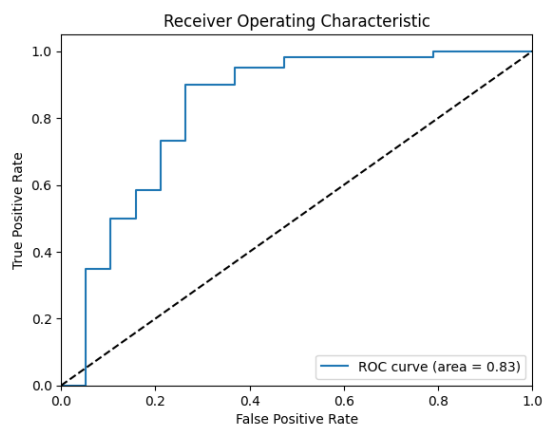


Fig. 3: Receiver operating characteristic (ROC) curve illustrating the trade-off between true positive rate and false positive rate for the classification model (AUC = 0.83)

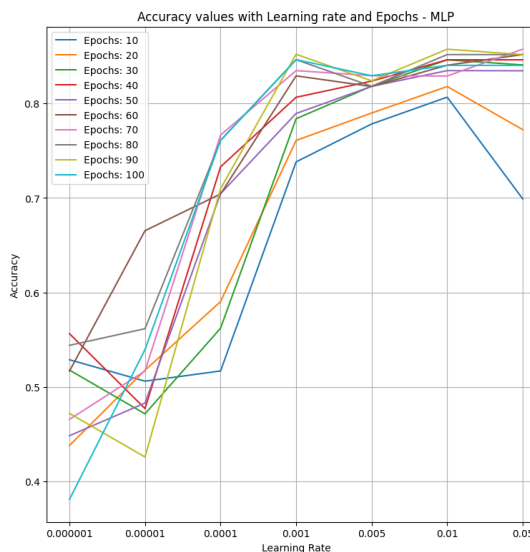


Fig. 5: The accuracy graph for MLP learning rate and epochs vs. accuracy

5 shows that the performance of the MLP model depends on the tuning of its hyperparameters, specifically the learning rate and number of epochs. The figure summarizes the training accuracy across different configurations and indicates that using a learning rate of 0.01 with 90 epochs produces the most stable and accurate results. This setting achieved an accuracy of 85.7% and provided more reliable classifications of ertapenem-resistant and susceptible *Klebsiella pneumoniae* compared to other tested configurations.

Random Forest classifiers were evaluated using different combinations of tree counts and maximum depths;

the optimal configuration produced a training accuracy of of 85.8%. Figure 6 shows that the performance of the Random Forest model depends on the hyperparameters used during tuning, particularly the maximum depth and number of estimators. The figure indicates that using a maximum depth of 7 with 90 estimators yields the highest accuracy of 85.8% and provided more reliable classifications of ertapenem-resistant and susceptible *Klebsiella pneumoniae* than the other tested settings.

The training phase produced strong accuracy across

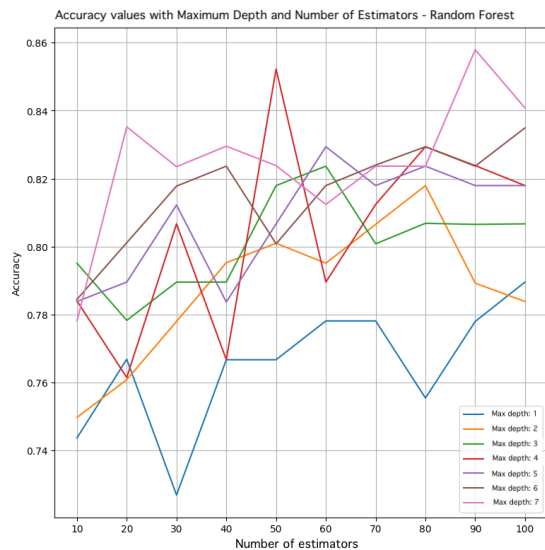


Fig. 6: The accuracy graph for Random Forest with maximum depth and the number of estimators vs. accuracy

the four models. Logistic Regression achieved a training accuracy of 83%, KNN reached 82%, Random Forest achieved 85.8%, and the MLP model performed at 85.7%. Based on overall accuracy and stability during training, Logistic Regression, Random Forest, and MLP were selected for final evaluation on the test dataset.

### B. Results at Model Testing Stage

In all confusion matrices and reported metrics, resistant (R) isolates were treated as the positive class. Figure 7 illustrates the normalized confusion matrix obtained from the Logistic Regression model on testing data. The confusion matrix presents classification performance between R and S. The model correctly classified 73.68% of those belonging to R, a true positive, and 90% for S, a true negative. Some resistant samples were predicted as susceptible, with 26.32% as a false negative, while a smaller number of susceptible samples were predicted as resistant, 10%, a false positive. The precision value was 0.81, the recall value was 0.82, the F1-score was 0.81, and the accuracy value was 0.86 (86%).

Figure 8 illustrates the performance of the MLP model for predicting ertapenem resistance in bacterial isolates is shown in with a normalized confusion matrix. The model was trained to classify isolates as either resistant or susceptible. The results indicate that the model accurately predicted 73.68 % of resistant samples, a true positive, and 86.67% of susceptible samples, a true negative. Some resistant samples were predicted as susceptible, with 26.32 % as a false negative, while a

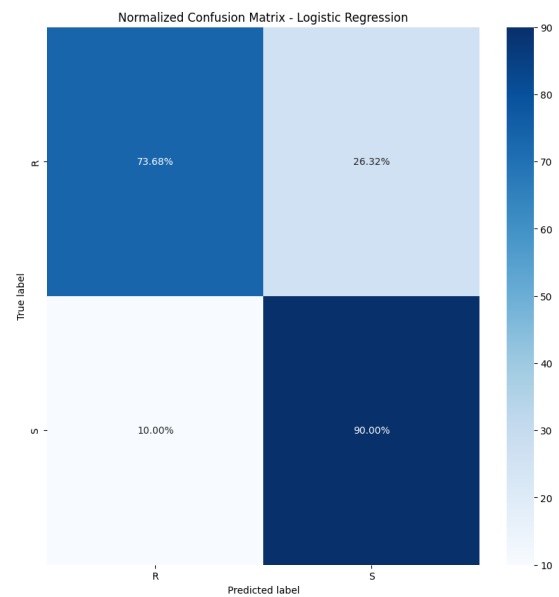


Fig. 7: Normalized confusion matrix showing the performance of the Logistic Regression model in predicting ertapenem resistance in bacterial isolate

smaller number of susceptible samples were predicted as resistant, 13.33%, a false positive. The precision value was 0.77, the recall value was 0.80, the F1-Score was 0.79, and the accuracy value was 0.84 (84%).

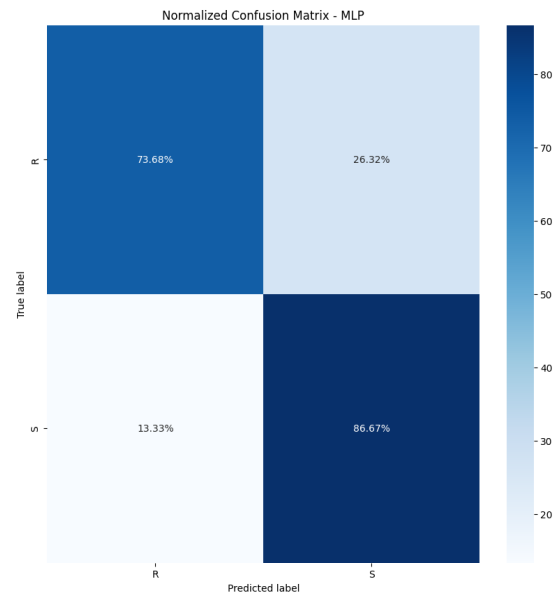


Fig. 8: Normalized confusion matrix showing the performance of the MLP model in predicting ertapenem resistance in bacterial isolate

Figure 9 illustrates the performance of the Random Forest model for predicting ertapenem resistance in

bacterial isolates is shown in with a normalized confusion matrix. The model was trained to classify isolates as either resistant or susceptible. The results indicate that the model accurately predicted 57.89% of resistant samples, a true positive, and 85% of susceptible samples, a true negative. Many resistant samples were incorrectly predicted as susceptible, 42.11%, a false negative, while a smaller number of susceptible samples were predicted as resistant 15%, a false positive. The precision value was 0.71, the recall value was 0.71, the F1-score was 0.71, and the accuracy value was 0.78 (78%).

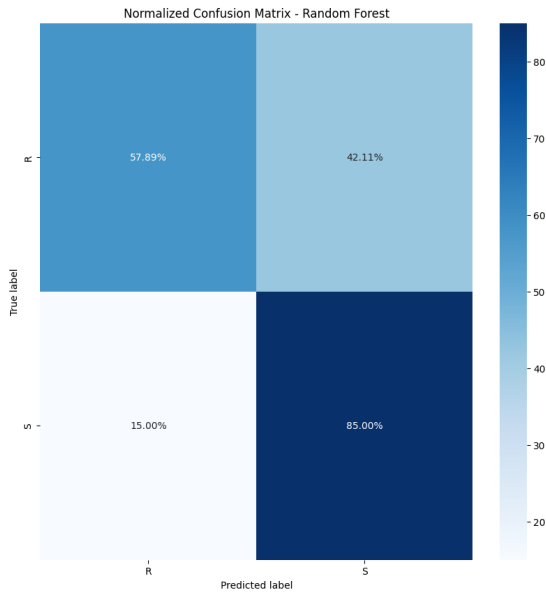


Fig. 9: Normalized confusion matrix showing the performance of the Random Forest model in predicting ertapenem resistance in bacterial isolate

TABLE I: Performance comparison of machine learning classifiers for predicting ertapenem resistance in *Klebsiella pneumoniae*

Algorithm	Precision	Recall	F1-Score	Accuracy
Logistic Regression	0.81	0.82	0.81	0.86
Random Forest	0.71	0.71	0.71	0.78
MLP	0.77	0.80	0.79	0.84

The result of these comparisons of precision, recall, and accuracy is summarized in Table I. The models tested to predict ertapenem resistance showed good performance overall. Logistic Regression achieved the most stable and accurate results, with a testing accuracy 86%. In contrast, the MLP and Random Forest models had moderate accuracy and some instability. Of all the models, Logistic Regression provided the best balance

between accuracy and consistency when classifying resistant and susceptible isolates.

TABLE II: Performance comparison of Data balancing experiments, showing Accuracy, Precision (macro and weighted), Recall (macro and weighted), and F1 Score (macro and weighted).

Experiment	Accuracy	Precision	Recall	F1-Score	Notes
Undersampling	0.86	0.81 / 0.86	0.82 / 0.86	0.81 / 0.86	26.32% FN
SMOTE	0.95	0.62 / 0.98	0.82 / 0.95	0.66 / 0.96	68.42% FN
ADASYN	0.95	0.68 / 0.98	0.79 / 0.95	0.64 / 0.96	36.84% FN
Class-Weighting	0.99	0.99 / 0.99	0.63 / 0.99	0.70 / 0.98	73.68% FN

While balancing techniques increased overall accuracy, these gains were accompanied by a marked rise in false-negative predictions for the resistant class. Undersampling the majority class achieved the highest macro recall (82%) and macro F1-Score (81%) and the lowest rate of resistant isolates misclassified as susceptible (26.32%), indicating superior clinical reliability. In contrast, SMOTE, ADASYN, and class-weighting approaches produced higher accuracy but significantly poorer recall for resistant isolates, with false negative rates exceeding 36%, and reaching 73.68%. These findings demonstrate that accuracy alone is insufficient for evaluating antimicrobial resistance prediction models and underscore the importance of prioritizing recall and error asymmetry.

## V. CONCLUSION

This study demonstrates that ertapenem resistance in *Klebsiella pneumoniae* can be predicted from MALDI-TOF MS spectra using supervised machine learning. By concentrating on ertapenem specifically, the work addresses a gap left by earlier studies that examined carbapenem resistance more generally or relied on deep learning models that are not practical for routine laboratory use. In contrast, this project evaluates simpler models that can run on standard laboratory computers and still perform well. Among them, Logistic Regression produced the strongest and most stable results, with an accuracy of 86% and an F1-score of 0.81. These findings show that resistance-related signals are present in MALDI-TOF spectra and can be captured using accessible analytical methods.

Focusing on ertapenem enhances the clinical relevance of the predictions since ertapenem resistance often appears before resistance to other carbapenems, giving clinicians an early indication that a *Klebsiella pneumoniae* strain may be broadly drug-resistant. In addition, ertapenem is commonly used in clinical practice due to its once-daily dosing, ease of administration, and

lower cost compared with other carbapenems. These characteristics make it both clinically important and a practical target for focused resistance prediction.

The study also introduces a structured preprocessing workflow that improves spectral quality and helps the models detect subtle differences between resistant and susceptible isolates. MALDI-TOF MS is already widely used for rapid bacterial identification, so integrating a predictive layer into existing workflows could provide clinicians with early resistance insights while they wait for culture-based results. Future studies should expand the dataset to include isolates from additional hospitals and geographic regions to improve generalizability and reduce site-specific bias. It would also be valuable to explore how MALDI-TOF MS features relate to results from traditional susceptibility testing to better understand the biological basis of the spectral patterns. Despite these limitations, this study offers a focused and practical framework for ertapenem resistance prediction using MALDI-TOF MS data. With further validation, this approach has the potential to support earlier antimicrobial resistance detection and help clinicians make more timely and informed treatment decisions.

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