

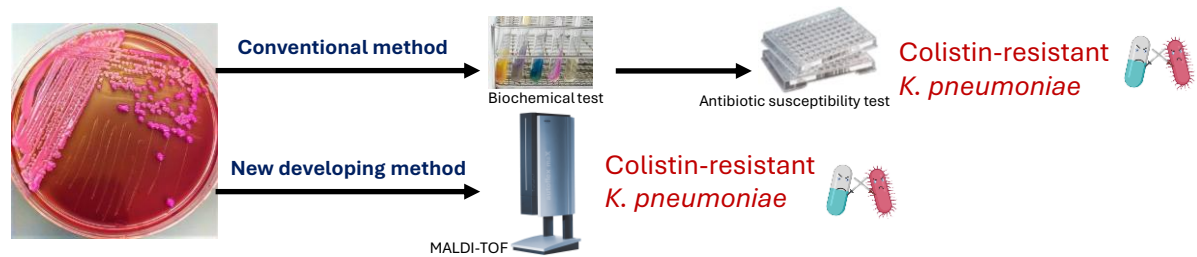
## INTRODUCTION

**Colistin-resistant *Klebsiella pneumoniae* (ColR-KP)** refers to strains that are resistant to colistin, a last-resort antibiotic used for treating severe, multidrug-resistant infections, particularly those caused by carbapenem-resistant *K. pneumoniae*. The emergence of CRKP poses a critical public health threat due to severely limited therapeutic options and high patient mortality rates. Early and accurate detection of colistin resistance is crucial for effective antimicrobial stewardship and managing potentially life-threatening infections.

**Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)** can be a valuable tool for rapid identification of colistin-resistant *Klebsiella pneumoniae* (ColR-KP). This technology allows for quick detection of resistance patterns, potentially guiding timely and appropriate antibiotic treatment.

### Objectives

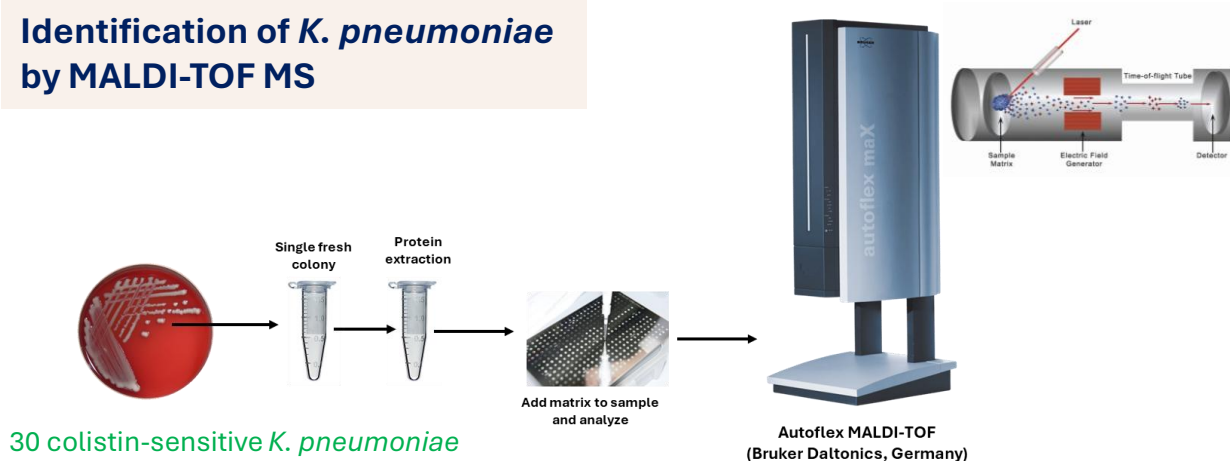
- To analyze protein pattern of colistin resistant *K. pneumoniae* comparing with colistin sensitive *K. pneumoniae*
- To identify protein biomarkers for the differentiation of colistin-resistant and colistin-sensitive *K. pneumoniae*.



## MATERIALS AND METHODS

In this study, we investigated the protein patterns of *K. pneumoniae* by analyzing 30 colistin-sensitive and 30 colistin-resistant isolates using MALDI-TOF MS.

### Identification of *K. pneumoniae* by MALDI-TOF MS



### MALDI-TOF MS data analysis

60 spectra  
- Colistin resistant KP = 30 spectra  
- Colistin susceptible KP = 30 spectra

#### Preprocessing step

1,095 raw peaks

Feature selection using DT algorithm

Identify peaks that have relative intensity expression differences among the 2 groups

Construct the DT model for prediction

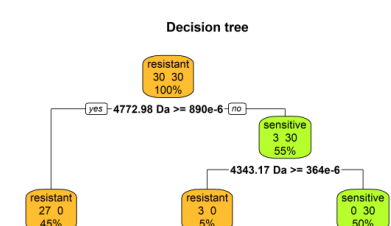


| Clear identification results display at species level |           |                              |              |
|---|-----------|------------------------------|--------------|
| Sample name   | Sample ID | Organisms                    | Score values |
| A1  | 25689     | <i>Klebsiella pneumoniae</i> | 2.98         |
| A2  | 25690     | <i>Klebsiella pneumoniae</i> | 2.75         |
| A3  | 25691     | <i>Klebsiella pneumoniae</i> | 2.65         |
| A4  | 25692     | <i>Klebsiella pneumoniae</i> | 2.59         |
| A5  | 25693     | <i>Klebsiella pneumoniae</i> | 2.52         |

Compass software automatically generates MALDI-TOF Spectrum

#### Clustering analysis

#### Decision-tree (DT) algorithm in the rpart R package



#### Biomarkers identification

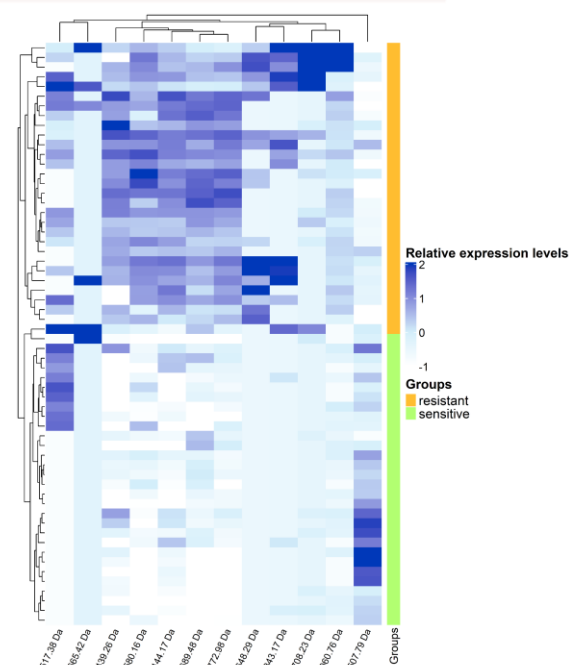
## RESULTS AND DISCUSSIONS

### Identification of *K. pneumoniae* isolates by MALDI-TOF MS

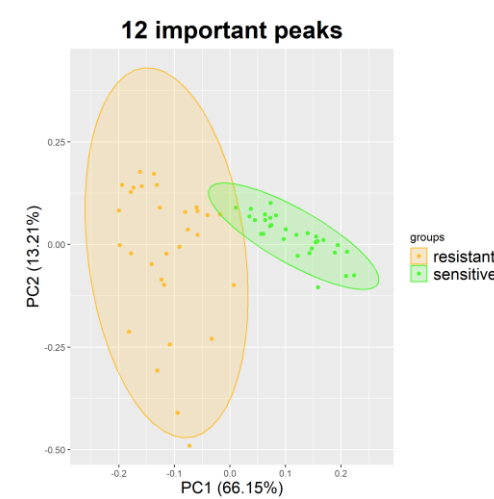
- A total of 60 isolates were correctly identified as *K. pneumoniae* by MALDI-TOF.
- 30 of *K. pneumoniae* isolates are colistin resistant and other 30 isolates are colistin susceptible.

### Clustering differentiation based on MALDI-TOF protein profiles

- Of the 1095 raw peaks detected, twelve were identified as significant



**Figure 1** Heatmaps representing 12 importance peaks. Color intensity indicates relative expression level between the importance peaks and each groups.



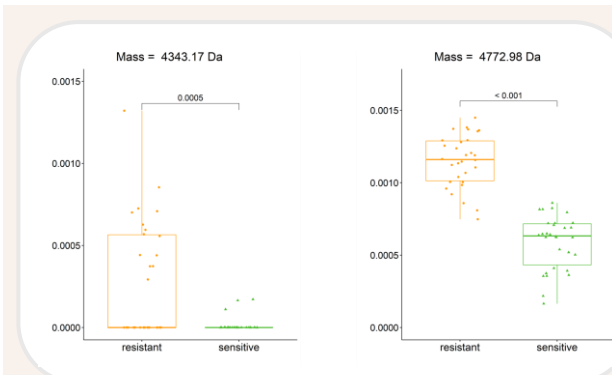
12 important variable peaks  
 "4772.98 Da"  
 "5144.17 Da"  
 "2689.48 Da"  
 "3580.16 Da"  
 "4060.76 Da"  
 "4439.26 Da"  
 "4343.17 Da"  
 "4708.23 Da"  
 "2507.79 Da"  
 "2517.38 Da"  
 "7648.29 Da"  
 "8665.42 Da"

**Figure 2** PCA analysis showed a distinction of importance peaks clustered in two main groups: resistant (yellow) and sensitive (green)

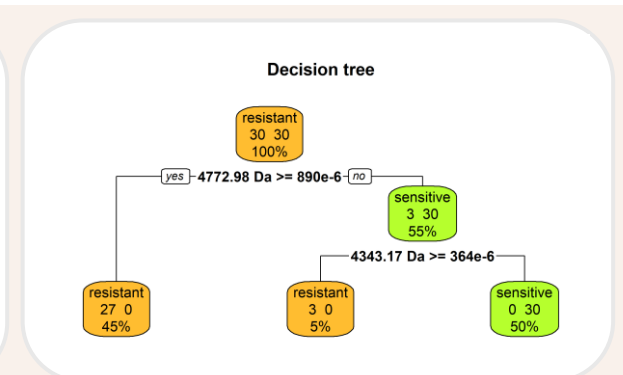
- These twelve peaks were further analyzed using Principal Component Analysis (PCA), and the peaks at **4772.98 Da** and **4343.17 Da** were identified as potential biomarkers for discriminating between colistin-resistant and colistin-sensitive *K. pneumoniae*.

### Protein biomarker

- Decision tree** for identification of Numbers in each box represent numbers of spectra.
- The percentage inside each box represents the proportion of spectra that can be classified using each peak marker.



**Figure 3** Box plot of relative intensity of protein biomarkers. The statistical difference in intensity among the groups we were analyzed by Dunn's test.



**Figure 4** Decision tree for identification of ColSKp (green), ColRKp (yellow).

## CONCLUSION

- This study provides significant protein biomarkers that allow for the differentiation of colistin-resistant and colistin-sensitive *K. pneumoniae*.
- This information could aid in the future development of rapid identification methods for CRKP

## ACKNOWLEDGEMENT

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