

# Rapid identification of *Klebsiella pneumoniae* and *Serratia marcescens* by surface-enhanced Raman spectroscopy

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**Abstract.** Two types of pathogenic bacteria, *Klebsiella pneumoniae* and *Serratia marcescens*, had been reported as important causes of hospital-acquired infection. Rapid and accurate identification of *Klebsiella pneumoniae* and *Serratia marcescens* is vitally important for the selection of appropriate treatment modalities. In this article, the feasibility of using surface-enhanced Raman Spectroscopy (SERS) to identify *Klebsiella pneumoniae* and *Serratia marcescens* was explored. Spectrum samples were obtained from *Klebsiella pneumoniae* infections (n=1000) and *Serratia marcescens* infections (n=1000). The differences between the spectra of two types of pathogenic bacteria were also analyzed. Moreover, Principal Component Analysis-Linear Discriminant Analysis (PCA-LDA) algorithm was used to discriminate the spectra of pathogenic bacteria.

## 1. Introduction

Bacterial infection is regarded as a leading cause of death globally [1]. There were about 13.7 million deaths associated with bacterial pathogens worldwide in 2019 [2]. *Klebsiella pneumoniae* and *Serratia marcescens* are considered as two of the most common pathogenic bacteria, which serious threat to the health of global publication [2]. As gram-negative bacterium, *Klebsiella pneumoniae* and *Serratia marcescens* can cause multiple diseases, including pneumonia, septicemia and community-acquired meningitis, et al. [3,4]. The ability to rapidly identify pathogenic bacteria is important for understanding pathogenicity of specific strains, and performing traceability analysis. Polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA) were common methods for detecting pathogenic bacteria, which were limited by significant time, complex preparation procedures, and professional staff [5]. Therefore, quick and accurate identification of pathogenic bacteria have great requirement.

Raman spectroscopy, relying on inelastic scattering of photons, attracts great interest for biomedical analysis, especially for cancer screening [6-8]. Currently, SERS also were used for detection of pathogenic bacteria. Chen et al. used SERS to distinguish between methicillin-resistant staphylococcus aureus and other six standard strains [9]. Zhang et al. successfully discriminated *Escherichia coli* and *Staphylococcus aureus* based on dual recognition by vancomycin and aptamers using SERS method [10].

In this article, the feasibility of distinguishing *Klebsiella pneumoniae* and *Serratia marcescens* with SERS was investigated. Furthermore, the differences of SERS spectra achieved from *Klebsiella pneumoniae* and *Serratia marcescens* were also compared using PCA-LDA.

## 2. Materials and methods

### 2.1. Bacterial culture

Blood agar plates were sealed with Parafilm and stored at 4°C for four hours. *Klebsiella pneumoniae* and *Serratia marcescens* isolates were cultured on these plates, which were obtained from shengli clinical college of Fujian medical university.

### 2.2. SERS measurements

For measurement, 3  $\mu\text{L}$  of bacterial suspension ( $10^8\text{cfu/mL}$ ) was prepared, and dried on gold-plated silica glass for one hour. The SERS spectra were obtained from a Renishaw inVia confocal Raman microscope with a 633 nm diode laser and a  $100\times$  objective (NA: 0.9). The spectral range was from 380 to  $1790\text{cm}^{-1}$ , and the integration time was 1s. Five points were randomly selected from each sample for spectrum measurement. The auto-fluorescence background was subtracted from the raw spectra by Vancouver Raman algorithm [11].

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### 3. Result

#### 3.1. Comparison of SERS spectra

An initial attempt was to explore the differences of SERS spectra from *Klebsiella pneumoniae* infections (n=1000) and *Serratia marcescens* infections (n=1000) in peak position and peak intensity. The mean and difference SERS spectra of two pathogenic bacteria were showed in Figure 1.

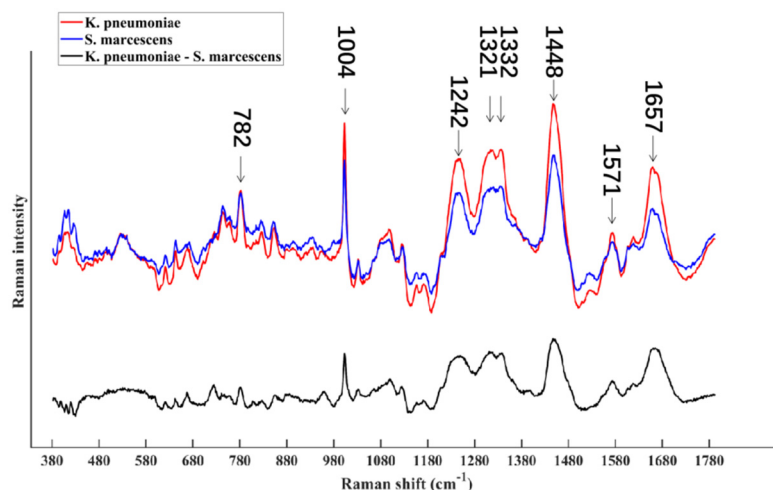


Figure 1 Mean and difference SERS spectra from *Klebsiella pneumoniae* and *Serratia marcescens*.

As can be seen in Figure 1, both SERS spectra of *Klebsiella pneumoniae* (red line) and *Serratia marcescens* (blue line) show similar profile in terms of peak position and peak intensity. The intensity differences of the mean spectra from *Klebsiella pneumoniae* and *Serratia marcescens* were mainly reflected at 782, 1004, 1242, 1321, 1332, 1448, 1571 and 1657  $\text{cm}^{-1}$ , respectively. In addition, for *Klebsiella pneumoniae* the peak intensity of 782, 1004, 1242, 1321, 1332, 1448, 1571 and 1657  $\text{cm}^{-1}$  were higher than that of *Serratia marcescens*. The tentative assignments for main SERS peaks are listed in Table 1 according to previous reports [12].

Table 1 SERS peak positions and tentative assignments

| Peak positions | Tentative assignments          |
|----------------|--------------------------------|
| 782            | Lactose                        |
| 1004           | L-Phenylalanine                |
| 1242           | Acetyl coenzyme A              |
| 1321           | L-Valine                       |
| 1332           | Adenine                        |
| 1448           | 15-Methylpalmitic acid (17iso) |
| 1571           | L-Histidine                    |
| 1657           | Malic acid                     |

#### 3.2. Multivariate analyses of SERS spectra

The two SERS spectra were too similar to be used to directly distinguish *Klebsiella pneumoniae* and *Serratia marcescens*. The feasibility of using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to improve the classification of SERS spectra between two groups was tested. In brief, a total of 2000 samples were randomly divided to two parts. The training group was composed of 900 samples of *Klebsiella pneumoniae* and 900 samples of *Serratia marcescens* and the remaining 200 samples were used for testing. PCA was used to reduced the dimension of samples, and the three principal components were extracted as input of LDA classifier for distinguishing *Klebsiella pneumoniae* from *Serratia marcescens*. The PCA-LDA algorithm performed well not only in training group but also in test group. For test group, the high diagnostic accuracy of 91.5%, sensitivity of 100% and specificity of 85.47% were achieved from the confusion matrix, as showed in figure 2.

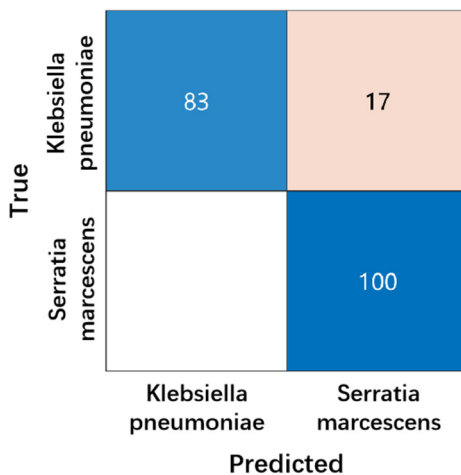


Figure 2 Confusion matrix of PCA-LDA classification.

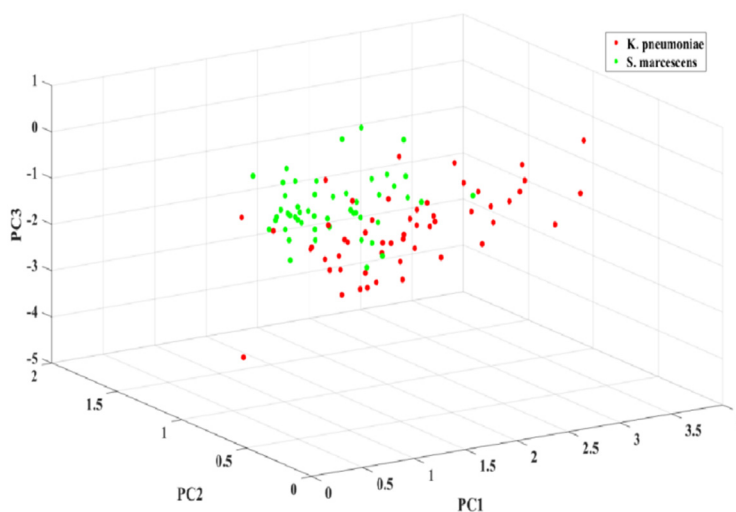


Figure 3 Classification results of the PCA-LDA model in the 3D feature space.

Furtherly, the classification results of the PCA-LDA model in the 3D feature space were showed in Figure 3. The red dots represent Klebsiella pneumoniae samples, the green dots represent Serratia marcescens samples. As showed in Figure 3, two pathogenic bacteria could be well distinguished by the PCA-LDA training. The scatter plot

of the posterior probability values for Klebsiella pneumoniae and Serratia marcescens using PCA-LDA, and receiver operating characteristic (ROC) curves were showed in figure 4.

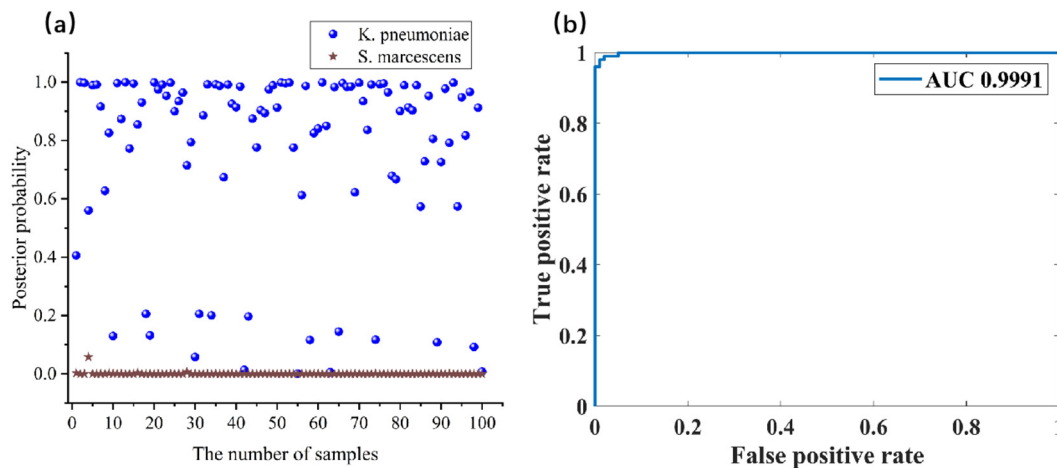


Figure 4 (a) Scatter plot of the posterior probability values and (b)Receiver operating characteristic (ROC) curves.

## 4. Conclusion

In conclusion, there were differences in the peak intensity of SERS spectra between *Klebsiella pneumoniae* and *Serratia marcescens* could be observed. These differences indicated that SERS spectra have great potential for identification of *Klebsiella pneumoniae* and *Serratia marcescens*. The results from PCA-LDA reinforced this view. Our experiments pointed SERS may be a faster and effective method to directly detect pathogenic bacteria in foods and clinical samples. Further research will be done about antibiotic resistance in *Klebsiella pneumoniae* and *Serratia marcescens* using label-free Raman spectroscopy.

## Acknowledge

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