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Rapid typig of ciprofloxacin non-susceptible Klebsiella pneumoniae strains by using the Raman spectrum-based machine-learning algorithm

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Background: The emergence and spread of antibiotic resistant *Klebsiella* are great concern. The U.S. National Healthcare Safety Network (NHSN) reports that gram-negative bacteria are responsible for more than 30% of hospital-acquired infections. Among them, *K. Pneumoniae* is regarded as one of the most important nosocomial pathogen and the increase of resistance to antimicrobial agents are resulting limitations on the therapeutic options. Rapid and simple identification method of antibiotic resistant strains are required for the optimal decision making for patients. We introduce a label-free quantization method to classify subtypes of quinolone-resistant *K. pneumoniae* isolates obtained from human blood cultures.

Material/methods: The Raman spectra of *K. pneumoniae* were obtained through a SENTERRA confocal Raman system (Bruker Optics, Billerica, MA). Self-made gold surface-enhanced Raman scattering (SERS) substrates were prepared to evaluate the Ciprofloxacin (CIP)-susceptible *K. pneumoniae* ATCC70063 and two multilocus sequencing typing (MLST)-characterized CIP non-susceptible strains such as, *K. pneumoniae* ST11 and *K. pneumoniae* ST15. CIP non-susceptible *K. pneumoniae* isolates were collected from blood cultures at Kyung Hee University Hospital, in Seoul, South Korea. Since all bacteria cultured were transferred to aquatic conditions through distilled water, there was a coffee-ring effect in each specimen dropped on SERS substrate. Bacterial drop coating deposition method was used. Therefore, the central and ring zones of each *K. pneumoniae* strain were measured to detect the ideal zone to *K. pneumoniae* isolates.

Results: The ring zone-acquired spectra showed prominent Raman peaks at 853 cm–1 (tyrosine ring breath), 1002 cm–1 (C–C aromatic ring breathing stretching vibrational mode of phenylalanine), 1240–1260 cm–1 (amide III β-sheet), 1445 cm–1 (C–H2 scissoring deformation vibrational mode), and 1665 cm–1 (amide I α-helix) while the central zone-acquired spectra showed strong Raman peaks at 1002, 1240–1260, 1445 cm–1, and 1665 cm–1. It is impossible to visually classify three MLST-characterized *K. pneumoniae* strains via threshold value (THV)-decided biomarkers; however a support vector machine learning algorithm-assisted principal component analysis (PCA) method achieved a superior performance in not only classifying similar microbes, such as quinolone-resistant *K. pneumoniae* ST11 and *K. pneumoniae* ST15 strains, but also detecting the presence of quinolone-resistant *K. pneumoniae* stain. Particularly, the central zone led to higher sensitivity and selectivity compared to the ring zone.

Conclusion: This label-free, machine-learned, nondestructive screening method is likely to be useful for rapid typing of quinolone-resistant *K. pneumoniae* stains when used with a multivariate statistics-preprocessed machine learning-judged bio-classification system.

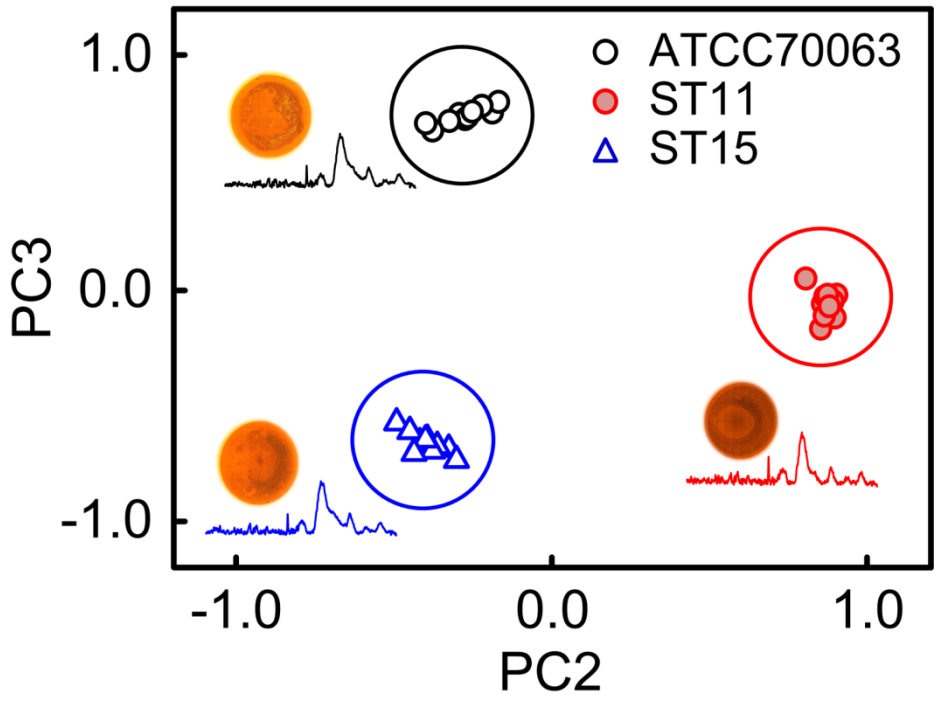

Figure 1. PCA score plot of PC2 versus PC3 for classifying two *K. pneumoniae* antibiotic resistant strains and ATCC70063. Morphologies and Raman spectra of three-type *K. pneumoniae* bacterial fluids deposited on SERS substrate