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Abstract

Introduction

Antibiotic resistance is a critical global health concern, necessitating innovative approaches for rapid antibiotic susceptibility testing (AST).

Raman spectroscopy, a label-free, non-destructive technique, offers a promising solution by detecting biochemical changes in bacteria. This study explores the use of Raman spectroscopy to assess the susceptibility of *Escherichia coli* (*E. coli*) to gentamicin and *Staphylococcus aureus* (*S. aureus*) to tigecycline across multiple timescales.

Methods

Bacterial cultures of *E. coli* were exposed to varying concentrations of gentamicin, while *S. aureus* cultures were treated with tigecycline. Each antibiotic was applied at sub-inhibitory, intermediate, and inhibitory concentrations for 1 hour, 4 hours, and overnight. Raman spectra were obtained for all treatment conditions, including controls (bacterial cells without antibiotics), capturing molecular fingerprints reflective of antibiotic-induced biochemical changes. Principal component analysis (PCA) was used to compare the spectra and identify patterns corresponding to bacterial susceptibility.

Results

Distinct spectral changes were observed in both bacterial species in response to antibiotic treatment. For *E. coli*, gentamicin exposure led to alterations in spectral peaks associated with nucleic acids and proteins, particularly at longer incubation times. Similarly, tigecycline-treated *S. aureus* showed spectral changes linked to ribosomal components and membrane lipids. PCA effectively differentiated treatment groups, with clear clustering patterns reflecting dose- and time-dependent effects. Overnight treatments consistently provided the most robust separation between susceptible and non-susceptible populations.

Conclusion

This study demonstrates the potential of Raman spectroscopy combined with PCA for rapid AST. The method successfully identified spectral biomarkers indicative of antibiotic susceptibility for two clinically relevant bacterial species. These findings support the development of Raman-based AST platforms to enhance clinical decision-making and combat antimicrobial resistance. Future work will explore the application of this approach to other antibiotics and bacterial strains to establish a comprehensive diagnostic framework.

Keywords

Raman Spectroscopy, Antibiotic susceptibility test