Rapid and Reagent-Free Method for Identification of Enterobacteriaceae Using Attenuated Total Reflectance Fourier Transformed Infrared (ATR-FTIR) Spectroscopy

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Introduction

• Fourier transform infrared (FTIR) spectroscopy is a reagent-free "whole-organism fingerprinting" technique that has been successfully employed for microbial identification in numerous research studies.

• The recent availability of portable FTIR spectrometers operating in the attenuated total reflectance (ATR) spectral acquisition mode enhances the simplicity, reliability, and practicality of implementing FTIR-based identification methods in routine clinical diagnostics.

• While traditional phenotypic methods for identification of enteric pathogens are time-consuming, ATR-FTIR spectroscopy can provide results within minutes after initial culture without the use of any reagents.

• We have developed an ATR-FTIR method for rapid identification of 7 clinically relevant genera in the family Enterobacteriaceae as well as for discrimination among E. coli pathotypes.

ATR-FTIR Spectroscopy

ATR-FTIR spectroscopy is similar in many respects to another "whole-organism fingerprinting" technique: MALDI-TOF MS. MALDI-TOF MS is based on measurement of infrared absorption by all biochemical cellular constituents (Figure 1) whereas MALDI-TOF MS is based on measurement of proteins in the range of 2-20 kilodaltons; primarily ribosomal proteins. Accordingly, ATR-FTIR spectroscopy can complement MALDI-TOF MS by providing additional discriminatory capabilities.

Figure 1. The spectrum of intact bacterial cells contains information on all their biochemical constituents and accordingly serves as a "whole-organism fingerprint".

Methods

- Scheme 1. Experimental Protocol for ATR-FTIR Spectral Acquisition

1. Culture on MacConkey agar plates (35°C, 18 h) and harvest 3 colonies
2. Collect 1-5 colonies on sterilized toothpick; apply onto ATR sampling surface (3-mm dia.)
3. Acquire FTIR spectrum (-1 min)
4. Clean ATR sampling surface with ethanol

- Minimal sample preparation, no chemicals required

The present study was done in a hospital bacteriology lab with a portable ATR-FTIR spectrometer (Agilent 5500 ATR-FTIR spectrometer, Agilent Technologies Inc.). A spectral database for use in identification of Enterobacteriaceae was created by acquiring the ATR-FTIR spectra of the clinical isolates described below. Bacterial colonies from culture plates were deposited on the ATR sampling surface of the spectrometer and their spectra immediately recorded to ensure that the microbial cells fully retained their water content. Spectra that failed to meet spectral quality criteria empirically developed in our prior work were rejected. All spectra were converted to first-derivative spectra in order to eliminate baseline variability and then vector-normalized. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) of the spectral data were performed employing SpectralAnalysis software (CogniSolve, Montreal, QC, Canada).

A key difference: ATR-FTIR spectroscopy is based on measurement of infrared absorption by all biochemical cellular constituents (Figure 1) whereas MALDI-TOF MS is based on measurement of proteins in the range of 2-20 kilodaltons; primarily ribosomal proteins. Accordingly, ATR-FTIR spectroscopy can complement MALDI-TOF MS by providing additional discriminatory capabilities.

Results and Discussion

- Novelty of the approach: To our knowledge, we are the only group to exploit the advantages of recently developed portable ATR-FTIR spectrometers for purposes of microbial identification. In our previous work, we demonstrated the potential utility of this technology for the rapid identification of MRSA and VRE. In the present study of Enterobacteriaceae, ATR-FTIR spectra were acquired in triplicate by taking different colonies from the same agar plate. Based on the application of spectral quality criteria, on the order of 5% of the spectra were rejected, resulting in a spectral database size of ~1300 spectra.

Classification by HCA: For four of the seven genera in the database, more than one species was included; however, classification of the ATR-FTIR spectra at the species level was restricted to species represented by at least 20 isolates. HCA and PCA were both employed to develop classification models with the use of a spectral-region selection algorithm that maximizes separation of classes based on the selection of discriminatory spectral features. Dendrograms generated by HCA of the first-derivative ATR-FTIR spectra of the clinical isolates based on the spectral regions selected in the optimization of the classification models are presented in Figure 2. A single model allowed for correct genus-level classification of all clinical isolates; however, the two models represented in Figure 2 were required for correct species-level classification, where applicable.

Figure 2. Dendrograms based on hierarchical cluster analysis of ATR-FTIR spectra of all clinical isolates in the Enterobacteriaceae spectral database.

- The clear separation of E. coli and Shigella spp. shown above is noteworthy, in light of their genetic similarity. The capability of ATR-FTIR spectroscopy to differentiate between these two groups is further illustrated in Figure 3, which shows the PCA results obtained for differentiation between E. coli and all four species of Shigella. It may also be noted that this capability is not provided by MALDI-TOF MS.

Figure 3. Principal components scores plot from PCA of the ATR-FTIR spectra of clinical isolates illustrates the capability of ATR-FTIR spectroscopy to discriminate between E. coli and Shigella spp.

Figure 4 presents a dendrogram generated by PCA of the first-derivative spectra of the 96 pathogenic E. coli strains in our ATR-FTIR spectral database for which pathotypes and serotypes were known, illustrating complete differentiation of the pathotypes based solely on their spectral profiles.

Figure 4. Dendrogram depicting the differentiation among E. coli pathotypes based on hierarchical cluster analysis of ATR-FTIR spectra for purposes of illustration, the size of the dendrogram has been reduced by grouping the spectra acquired for each pathotype into several subtypes.

In order to evaluate the potential of this approach for pathotype identification, the ATR-FTIR spectra of 20 pathogenic E. coli strains were acquired in a blind validation study and classified by pathotype based on similarity to the spectra in the ATR-FTIR database. All 20 strains were correctly classified.

Conclusions

The portable ATR-FTIR spectrometer and the simple experimental protocol employed in this study yielded highly reliable FTIR data.

High rates of correct classification of 7 genera of Enterobacteriaceae were achieved by multivariate statistical analysis of the ATR-FTIR spectra of a large set of clinical isolates.

By truly performing "whole-organism fingerprinting", ATR-FTIR spectroscopy complements MALDI-TOF MS, providing sub-species discrimination as well as the capability to differentiate between E. coli and Shigella.