



Raman spectroscopy for rapid subspecies identification of *Staphylococcus aureus*

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Introduction

We are developing Raman spectroscopy as a rapid typing-tool for use in clinical microbiology.

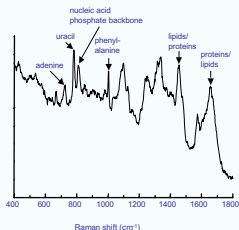
Here we have analyzed a collection of MRSA isolates that have been analyzed previously using PFGE¹.

The set consisted of 20 isolates; 5 identical isolates (one PFGE type), 5 isolates that produced related PFGE subtypes, and 10 isolates each with a unique PFGE profile.

¹ Van Belkum, A., et al., J Clin Microbiol, 1998. 36(6): p. 1653-9.

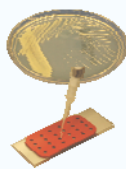
Raman spectroscopy

- a non destructive optical spectroscopic technique
- based on inelastic scattering of laser light by molecules
- Raman spectra reflect the molecular composition of a sample (i.e. they provide a spectroscopic fingerprint)

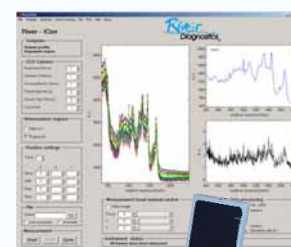


Raman spectrum of a bacterium with signal contributions of a number of cell constituents indicated

Method



- (1) standard overnight culture
- (2) transfer 1 μ L suspension (in water) on quartz slide
- (3) collect Raman spectrum on dedicated spectrometer (River Diagnostics)
 - 10-20s. signal collection
 - no dyes or labels



Results & Conclusion

Raman spectroscopy can distinguish between MRSA-isolates.

- Strains with identical PFGE-profiles are also indistinguishable by Raman
- Strains with related PGFE-profiles can be partially distinguished by means of Raman spectroscopy
- All 10 strains with unique PGFE profiles are distinguished by means of Raman spectroscopy.

These experiments were repeated on 3 different days and identical results here were obtained.

Work on other species is in progress.

Raman signal collection takes only 10-20s. and sample preparation is very simple, but yields results that are in line with genotyping methods.

We conclude that Raman spectroscopy is a rapid, powerful alternative typing method which paves the way for typing of isolates in clinical microbiology on a routine basis.

Top:

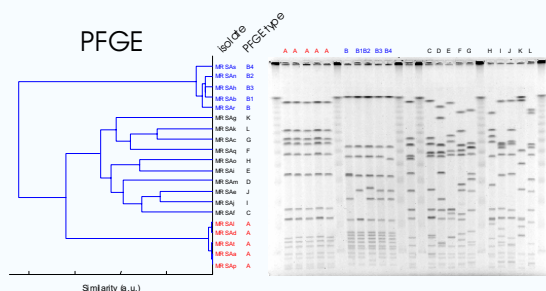
Pulsed Field Gel Electrophoresis (PFGE) analysis of MRSA isolates.

Hierarchical cluster analysis of PFGE profiles was performed using Dice correlation and Ward's clustering algorithm.

Identical PFGE profiles are indicated as type **A** (in red),

related PFGE profiles as type **B** with subtypes **B1-B4** (in blue).

PFGE



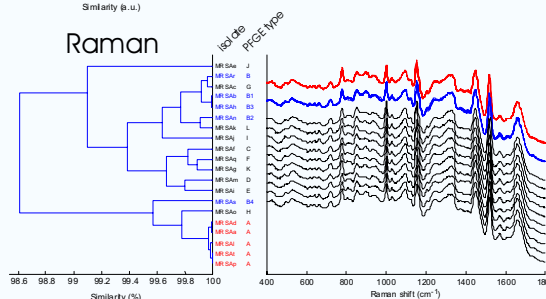
Bottom:

Raman spectra of MRSA isolates.

Hierarchical cluster analysis was performed using Euclidean distances and Ward's clustering algorithm.

PFGE types are also shown in the Raman dendrogram for comparison, using the same color coding as in the top figure

Raman



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