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EXTENDED ABSTRACTS

Tandem Mass Spectrometry Analysis as an Approach to Delineate Genetically Related Taxa, with Specific Implication for Differentiating Escherichia coli from amongst the Complex Enterobacteriaceae Family

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ABSTRACT

This work aims to guage the potential of GeLC-MS/MS to delineate taxa which are beyond the resolution of 16S rRNA and MALDI-TOF-MS identification, simultaneously discerning biomarkers of pathogenicity. 16S rRNA sequence analysis and MALDITOF- MS was performed to differentiate genetically closely related species from the Enterobacteriaceae . In parallel GeLC-MS/ MS, using E. coli and related enterobacteria as a model, was performed. Species specific peptides were identified and wont to create an optimised identification database, against which a panel of test strains were analysed to work out the resolution of GeLCMS/ MS for bacterial identification and strain characterisation. The panel included amongst others pathogenic E. coli strains, of differing pathotype, including E. coli O104:H4. The test strains might be resolved to the species and pathotype (subspecies) additionally , specific features might be identified. Using an optimised genome database and proteome profiling, we identified biomarkers that were specific for the test species and characterised strain-specific virulence factors. This proof of concept study demonstrates that whole genome sequences and GeLC-MS/ MS have the potential to both identify and characterise pathogenic bacteria during a single assay, introduction a replacement proteogenomic trend for microbial clinical diagnostics. Within the past decade, clinical microbiology laboratories experienced revolutionary changes within the way during which microorganisms are identified, moving faraway from slow, traditional microbial identification algorithms toward rapid molecular methods and mass spectrometry (MS). Historically, MS was clinically utilized as a high-complexity method adapted for protein-centered analysis of samples in chemistry and hematology laboratories. Today, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) MS is tailored to be used in microbiology laboratories, where it is a paradigm-shifting, rapid, and robust method for accurate microbial identification. Multiple instrument platforms, marketed by well-established manufacturers, are starting to displace automated phenotypic identification instruments and in some cases genetic sequence-based identification practices. This review summarizes the present position of MALDI-TOF MS in clinical research and in diagnostic clinical microbiology laboratories and is a primer to look at the “nuts and bolts” of MALDI-TOF MS, highlighting research related to sample preparation, spectral analysis, and accuracy. Currently available MALDI-TOF MS hardware and software platforms that support the utilization of MALDI-TOF with direct and precultured specimens and integration of the technology into the laboratory workflow also are discussed. Finally, this review closes with a prospective view of the longer term of MALDI-TOF MS within the clinical microbiology laboratory to accelerate diagnosis and microbial identification to enhance patient care. Across the world , the trend of the utilization of diagnostic MS methods is clear , and while laboratory scientists await FDA approval of the technology within the us , some are self-verifying the utilization of the technology. Sample preparation is both simple and reproducible. Most medical laboratory scientists can easily perform analysis of raw MS data and determine microbial identifications with the help of associated software. Finally, MS technology can interface directly with the laboratory data system (LIS) and reflex to other diagnostic testing. Thus, as MS continues to be implemented into modern clinical microbiology laboratories, it's important that laboratorians and clinicians alike become conversant in this paradigm-shifting technology. In short, MS technology is rapid, robust, customizable pursuant to the requirements of the laboratory, less expensive than current phenotypic testing methods despite the initial cost of the instrument, and, perhaps most significantly , easy to use. during this review, the mechanics and processes underlying MS for microbial identification are going to be described and demystified to form the technology more familiar and understandable .

Keywords: Mass spectrometry; Identification; GeLC-MS/MS; Pathotype; Enterobacteriaceae; Proteogenomic

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