Rapid Targeted LC-MS/MS Assay for ESKAPE Pathogen Identification Direct from Biological Fluids

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ACCEPTED ABSTRACT

INTRODUCTION: Microbial identification relies on bacterial culture followed by biochemical testing, which generally takes overnight. Protein analysis by MALDI-TOF MS has become a prominent player in clinical microbial diagnosis. Identification is nearly instantaneous, but requires overnight growth to produce the required pure colony. We recently published (1,2) a method for microbial identification direct from biofluid without the need for culture.

METHODS: Bacterial membrane glycolipids were extracted as published (1) and reconstituted in chloroform/methanol/water (3: 1.5: 0.25, v/v/v) for LC-MS/MS analysis on a SYNAPT G2 (Waters). Signature ions from each ESKAPE (*vide infra*) species were selected and fragmented. Suitable transitions were determined for each ESKAPE pathogens. The existing MALDI library (1) was used to comfirm LC-MS results after deconvolution and manual inspection used for polymicrobial analysis.

RESULTS: Previously we published use of microbial lipids for identification of bacteria and fungi direct from specimen using the Bruker Biotyper platform (1, 2). Here we present development of a pseudo-MRM assay for the ESKAPE pathogens (1) on a SYNAPT MS. A new extraction protocol that takes less than one hour is also introduced (5). Individual glycolipid preparations were analyzed by direct infusion to generate mass spectra for each ESKAPE species. Signature (lipid) ions, which are those unique to a given microbe, were selected for tandem MS fragmentation and their unique transitions, attributable to loss of various fatty acids, noted. For example, precursor ions at 1744, 1824, and 977 *m/z* (*z*=2) were found to be unique to *Enterobacter cloacae*. After successfully generating tandem mass spectra for each individual ESKAPE pathogen, mixed samples containing multiple pathogens were tested to demonstrate the method could distinguish and detect each organism in a simulated polymicrobial sample and where appropriate antibiotic resistance (3, 4).

CONCLUSIONS: ESKAPE pathogens, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*, were targted due to their high incidence of nosocomial infections and multidrug resistance. Here we present initial work to develop a rapid (< 1 hour (5)) pseudo-MRM assay targeted at detection of these microbes that are sensitive and resistant to antibiotic therapy.

NOVEL ASPECTS: Rapid identification of ESKAPE pathogens by LC-MS/MS points toward a targeted clinical assay that may be carried out on triple quadrupole systems rather than MALDI-TOF MS.

MOLECULES

Gram + Gram -

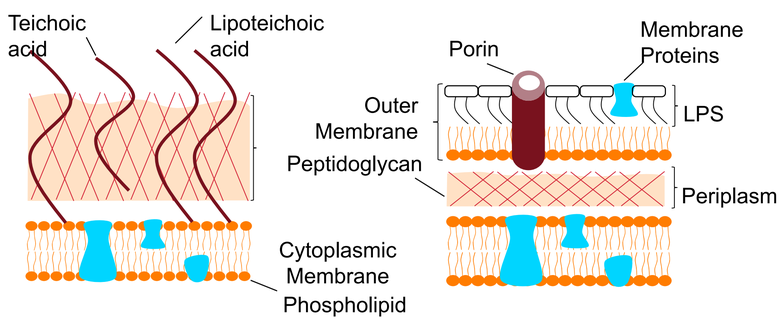


Figure 1. Bacterial membrane architecture. Cartoons of of Gram-positive (left) and Gram-negative (right) bacterial membranes that contain different classes of glycolipids, including lipopolysaccharide (LPS) in Gram-negative bacteria and cardiolipin (CL) in Gram-positive bacteria *vide infra*.

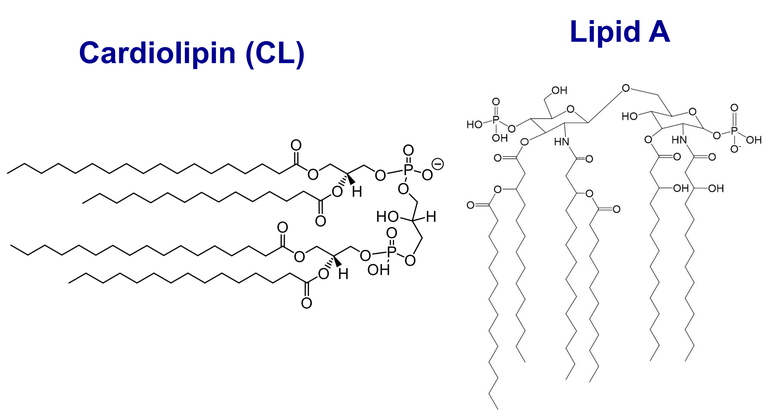
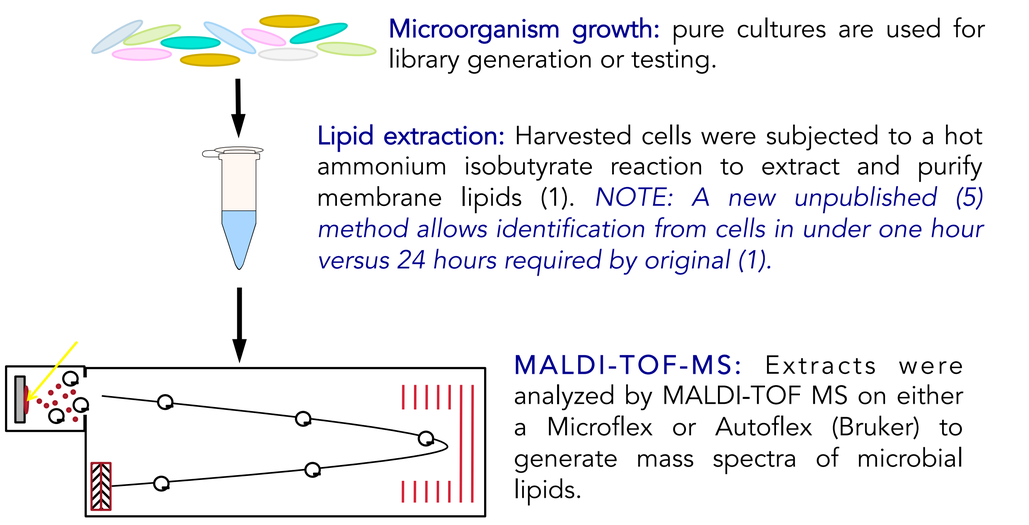


Figure 2. General chemical structures of Gram-negative LPS-derived lipid A (right) in *Escherichia coli* and Gram-positive derived cardiolipin (left) in *Staphylococcus aureus*. Microbial membrane lipids show structural variability that is species-specific and has been proposed as a means to discriminate between bacterial species (1). These lipids are readily extracted and visualized by mass spectrometry with structural differences resulting in unique mass spectral barcodes.

BACKGROUND

Our lipid-based strategy for bacterial identification utilizes the Bruker Biotyper platform and software (1). Libraries are constructed from lipid mass spectra as opposed to protein mass spectra.



Microorganism growth: pure cultures are used for library generation or testing.

Lipid extraction: Harvested cells were subjected to a hot ammonium isobutyrate reaction to extract and purify membrane lipids (1). *NOTE: A new unpublished (5) method allows identification from cells in under one hour versus 24 hours required by original (1).*

MALDI-TOF-MS: Extracts were analyzed by MALDI-TOF MS on either a Microflex or Autoflex (Bruker) to generate mass spectra of microbial lipids.

Library construction: Library was generated from high quality lipid mass spectra from cultured microorganisms. Mass spectral profiles from "unknown" samples were compared against our lipid library loaded into the Biotyper software platform (Bruker).

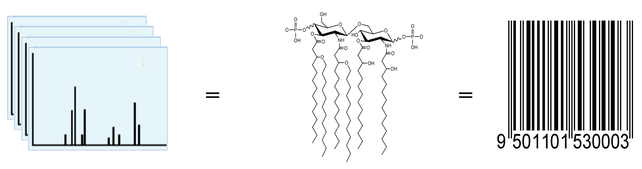


Figure 3: Strategy for lipid-based mass spectrometry platform for pathogen identification. A general workflow for the generation of mass spectra for development and validation of the glycolipid library.

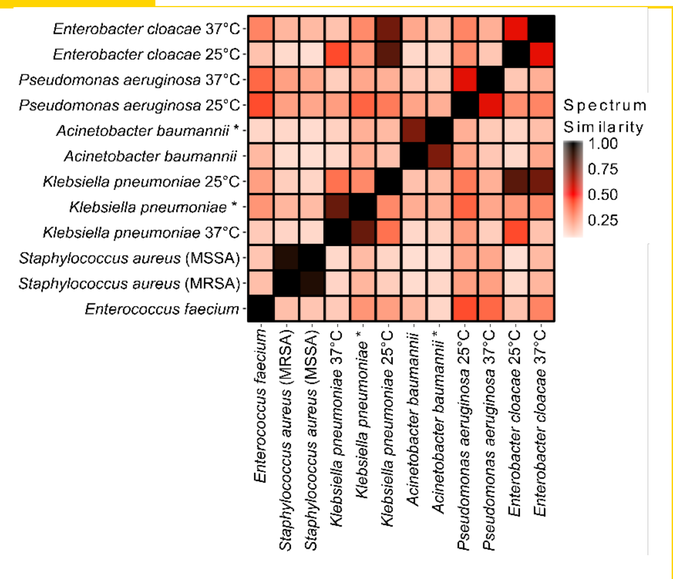


Figure 4. Dot product analysis of negative ion MALDI TOF mass spectral data for differentiation of ESKAPE pathogens. Results are represented as a heat map of comparisons of each mass peak pattern to itself and all others. A normalized similarity score of 1.0 is an identical match (black squares). A score of 0.0 where there is no match (white). (*) indicate colistin-resistant strains of *K. pneumoniae* and *A. baumannii*.

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RESULTS

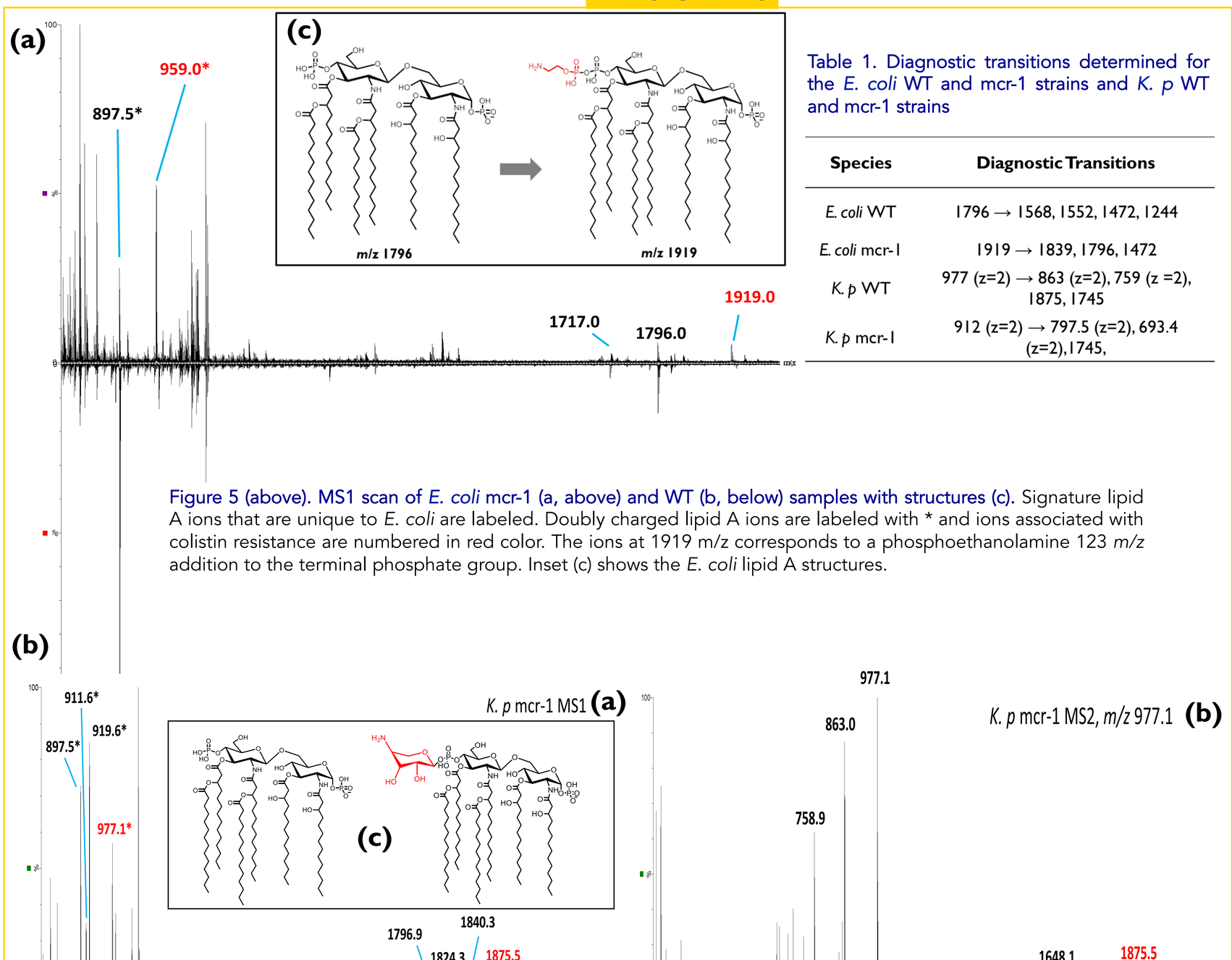

Figure 5 (above). MS1 scan of *E. coli* mcr-1 (a, above) and WT (b, below) samples with structures (c). Signature lipid A ions that are unique to *E. coli* are labeled. Doubly charged lipid A ions are labeled with * and ions associated with colistin resistance are numbered in red color. The ions at 1919 m/z corresponds to a phosphoethanolamine 123 *m/z* addition to the terminal phosphate group. Inset (c) shows the *E. coli* lipid A structures.

Table 1. Diagnostic transitions determined for the *E. coli* WT and mcr-1 strains and *K. p* WT and mcr-1 strains

Species	Diagnostic Transitions
E. coli WT	1796 → 1568, 1552, 1472, 1244
E. coli mcr-1	1919 → 1839, 1796, 1472
K. p WT	977 (z=2) → 863 (z=2), 759 (z =2), 1875, 1745
K. p mcr-1	912 (z=2) → 797.5 (z=2), 693.4 (z=2),1745,

Figure 6. (a) mass spectrum of K.p mcr-1 strain and (b) shows a tandem mass spectrum of lipid A ion at *m/z* 977.1. Doubly charged ions are observed and labeled with *. Red color numbers indicate colistin-resistant ions with an aminoarabinose (*m/z* 131) addition. Selected fragment ions are either loss of a fatty acid (*m/z* 977.1 → 863.0) or phosphate group. (*m/z* 977.1 → 1875.5). Inset (c) shows the *K. p* lipid A structures.

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