# Identification of the ESKAPE Pathogens by Mass Spectrometric Analysis of Microbial Membrane Glycolipids

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## Overview

Rapid pathogen identification is needed to allow physicians to respond to life-threatening infections. We propose essential membrane lipoglycans as a novel biomarker to expedite identification of pathogens by mass spectrometric analysis. Here we developed a database containing glycolipid mass spectra to identify the ESKAPE pathogens, *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.

## Background

- The membranes of the different microbial classes possess complex glycolipids, including lipopolysaccharide (LPS) in Gram-negative bacteria and cardiolipin (CL) in Gram-positive bacteria. (**Figure 1**)

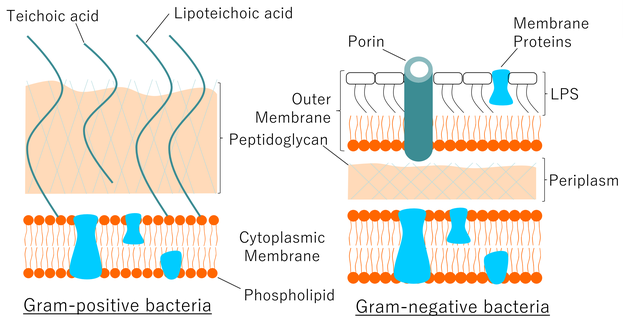
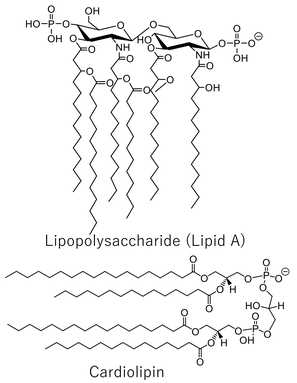



**Figure 1. General structure and key features of Gram-positive and negative bacterial membranes.**




- They show structural variability that is species-specific. (**Figure 2**)
- These glycolipids are readily extracted and visualized by mass spectrometry (MS) with the structural differences of these molecules resulting in a unique mass profile.

**Figure 2. General chemical structure of LPS-derived lipid A in *Escherichia coli* and cardiolipin in *Staphylococcus aureus*.**

## Funding

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## Methods

Our optimized strategy allowed for the multiplexed generation of a mass spectral database consisting of at least ten strains per ESKAPE pathogen and 44 additional microorganisms from diverse backgrounds.

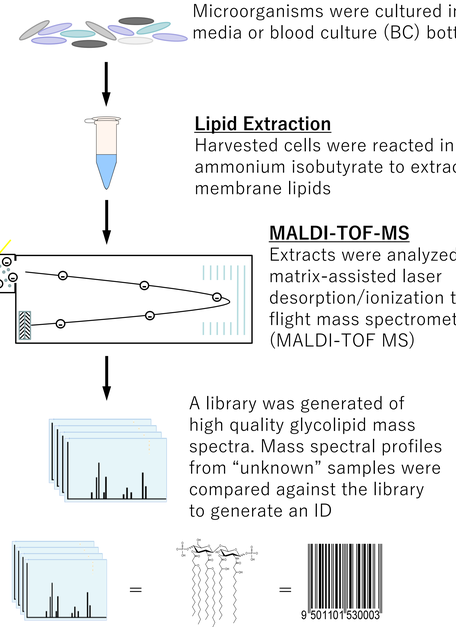


**Figure 3: A general workflow for development and validation of the glycolipid library.**

## Conclusions

- Dot products of mass spectra from the ESKAPE pathogens were able to differentiate by species.
- Consensus mass spectra reveal diagnostic ions for detecting colistin resistance in *A. baumannii* and *K. pneumoniae.*
- Dot products calculated between resistant and susceptible mass spectra achieve higher similarity scores when compared to the consensus spectrum with concurrent susceptibility.
- The mass spectral library was adapted to the MALDI Biotyper.
- Representative ESKAPE pathogens were detected from blood.

## Results - Dot product analyses of the ESKAPE pathogens determine differences in mass spectral patterns.

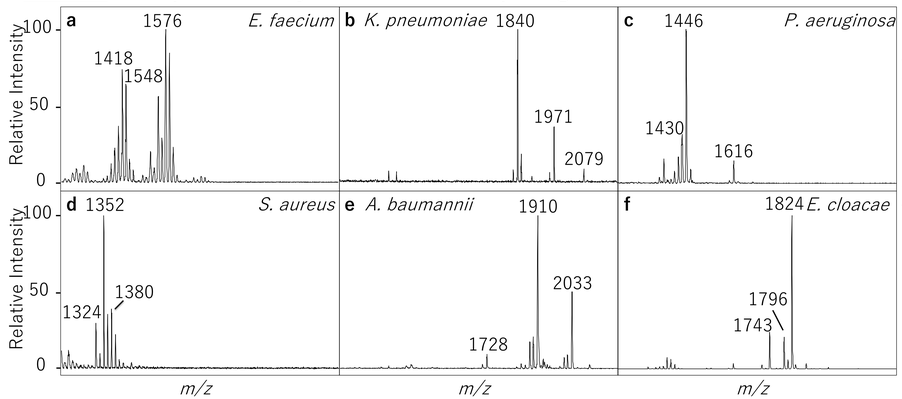


**Figure 4. Representative mass spectra from ESKAPE pathogens: (a) *Enterococcus faecium*; (b) *Klebsiella pneumoniae*; (c) *Pseudomonas aeruginosa*; (d) *Staphylococcus aureus*; (e) *Acinetobacter baumannii*; and, (f) *Enterobacter cloacae*.**

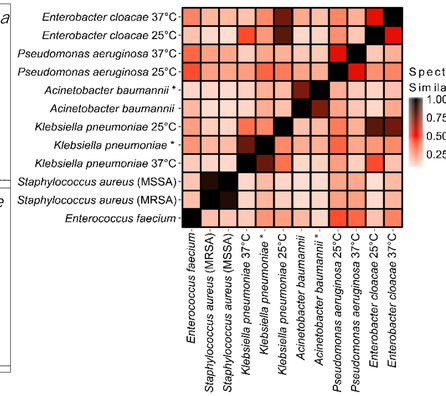


**Figure 5. Dot product analysis of the ESKAPE pathogens.** Results are represented as a heat map of comparisons of each mass peak pattern to itself and all others. (*) indicate colistin-resistant strains.

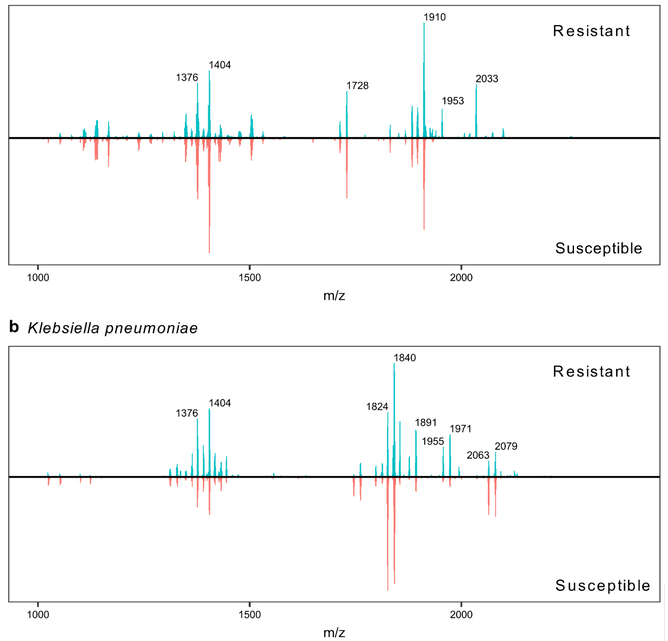


**Figure 5. Consensus mass spectra were created by summation of detected mass peaks from all replicates of colistin-resistant (top panels) and colistin-susceptible (bottom panels) *A. baumannii* (a) and *K. pneumoniae* (b).**

**Table 1. MALDI Biotyper results for identifying *K. pneumoniae* and *A. baumannii***

| Organism | | # strains | # replicates | Mean confidence log score | % positive sub-species identification | % positive species identification |
|---|---|---|---|---|---|---|
| *Klebsiella pneumoniae* | Colistin-susceptible | 26 | 96 | 2.528 | **100.0** | **100.0** |
| | Colistin-resistant | 34 | 220 | 2.536 | **69.2** | **87.7** |
| *Acinetobacter baumannii* | Colistin-susceptible | 188 | 555 | 2.270 | **55.9** | **77.2** |
| | Colistin-resistant | 25 | 93 | 2.615 | **92.3** | **92.3** |

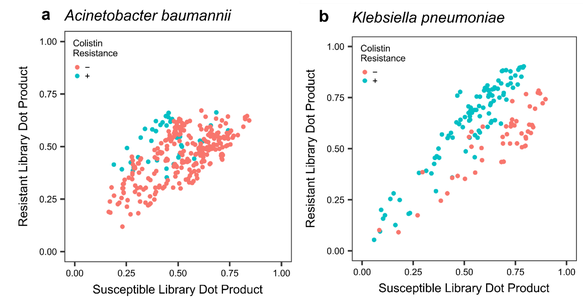


**Figure 7. Dot product scores between library consensus spectra and test spectra partitioned by colistin resistance for *A. baumannii* (a) and *K. pneumoniae* (b) replicates.**

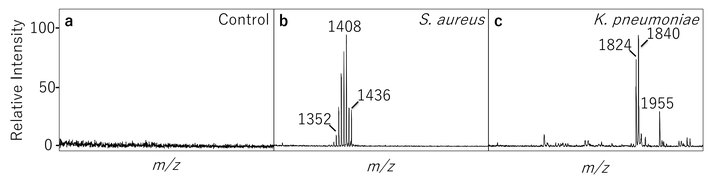


**Figure 8: Detection of ESKAPE pathogens in blood culture. (a.)** Sterile blood **(b.)** MRSA M2 after 24 hours, and **(c.)** *K. pneumoniae* B6 after six hours growth.