Detecting antibiotic resistance by MALDI-TOF analysis of bacterial membrane glycolipids

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Introduction

With the increasing prevalence of multidrug resistant Gram-negative bacteria, the last-line antibiotic, colistin, has gained importance resulting in the emergence of colistin-resistance in *Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*. These species are members of the ESKAPE pathogen family collectively showing increased resistance to antimicrobial agents. Mechanisms for resistance to colistin occur primarily through structural additions to lipid A, the lipophilic anchor of the essential bacterial membrane glycolipid lipopolysaccharide (LPS). These modifications serve to mask the electronegativity of lipid A and hinder binding by colistin and other cationic antimicrobial peptides. In this study, we analyze bacterial membrane glycolipids by MALDI-TOF-MS and develop methods to computationally detect colistin resistance in the ESKAPE pathogens.

Methods

Bacteria clinical isolates were cultured in lysogenic broth, harvested by centrifugation, and glycolipids were extracted using a hot ammonium-isobutyrate protocol. Mass spectra were acquired by MALDI-TOF-MS in negative ion reflectron mode on a MicroFlex LRF (Bruker) using a norharmane matrix. Mass spectra were analyzed and colistin-resistance models were built using the R statistical programming language.

Preliminary Data

Representative mass spectra from colistin-susceptible and -resistant strains of *Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* were analyzed for similarity by calculating a dot product. The dot product resulted in a value between 0 (least similar) and 1 (identical) representing similarity between two glycolipid mass spectra. This analysis revealed that there are detectable differences between colistin-susceptible and resistant *A. baumannii* and *K. pneumoniae*, which had dot products of 0.75 and 0.77, respectively. Based on these comparisons, we proceeded to develop machine-learning models of colistin resistance for individual species using *m/z* channels as features. Initial analysis of 141 *Klebsiella pneumoniae* samples (80 colistin-resistant and 59 colistin-susceptible) was performed by splitting samples into a training (50%) and test set (50%). A preliminary random forest model was trained with the training set and provided 94% accuracy in identifying colistin-resistant *Klebsiella pneumoniae* samples. Investigation of feature importance in this model revealed *m/z* 1972 as the top variable for discriminating colistin-resistant *Klebsiella pneumoniae.* Previous studies have identified *m/z* 1972 as resulting from an aminoarabinose addition to the phosphate of *Klebsiella pneumoniae* lipid A and have demonstrated its association with polymyxin resistance, supporting our approach. With this proof of concept, we aim to build a more comprehensive model with addition of multiple species.

Novel Aspect

We present methods to rapidly detect antibiotic resistance using lipid A.