Introduction

Acinetobacter baumannii, a nosocomial and multi-drug resistant pathogen, is responsible for 160,000 deaths worldwide. Morbidity and mortality of A. baumannii often take place in immunocompromised patients due to the development of multi-drug resistance (MDR) and ineffective antibacterial treatments leading to the rapid onset of sepsis, bacteremia and/or pneumonia.

Study Aims:
1. Characterize protein abundance differences between a non-MDR (19606) and an MDR (Ab5075) strain of Acinetobacter baumannii.
2. Determine proteome changes in the response of an MDR A. baumannii strain (Ab5075) to antibiotic treatment.

Strategy:
Protein Quantification

Network Generation

(Protein crosslinking)

Ab19606 Ab5075 (MDR)

Tandem mass tag (TMT) labeling

NHP-BDP and ReACT

Normalized protein abundances

Network of crosslinked protein interactions:

Quantitative Interaction Network

Methods

For crosslinking, biological replicates of Ab19606 or Ab5075 A. baumannii cultures were grown to stationary phase. For antibiotic treatment, Ab19606 cells were grown overnight in the presence of varying concentrations of antibiotic to stationary phase. Cells pellets were lysed by cryogenically, reduced, alkylated and digested with trypsin. Digested lysates were labeled with a TMT10 tag, followed by desalting and mass spectral analysis. TMT labeled peptides were loaded onto a 30 cm in-house pulled C18 reversed phase analytical column (Mag) and analyzed on an Orbitrap QEx (Thermo). Spectra were searched using Comet and ratios were quantified using Libra. Protein crosslinking experiments were carried out on living cells which were pulled C18 reversed phase analytical column (Mag) and analyzed on a Velos-FIT-ICR (Thermo) using ReACT (Windel et al. 2013). Crosslinked relationships were filtered to an FDR ≤ 5%, quantified peptides were filtered to an FDR ≤ 5%. Network analysis was performed using Cytoscape 3.0.

Discussion

Protein interaction reporter technology (XLI-M5) and quantitative analysis of multiple A. baumannii strains enabled determination of disparate abundances for clusters of protein interactions across strains. By mapping quantified protein abundances differences between Ab19606, a MDR strain, and Ab5075, an MDR strain, we determined clusters of nodal interactions that correlate with an MDR phenotype. Increased expression of Oxa-23, a beta-lactamase, and various efflux pumps are known to be highly expressed in MDR strains. Analysis of protein interactions from A. baumannii additionally revealed hypothetical proteins with significant abundance differences across strains that interacted directly with these proteins establishing novel, putative virulence factors within these MDR interaction clusters. Protein abundances were also analyzed as a function of antibiotic treatment of Ab5075 to identify bacterial response to therapeutics.

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References