IPD Project Details

Project ID: IPD5407

Project Title: Proteomic profiling of Klebsiella pneumoniae resistant against gradual increasing dose of colistin and meropenem

Description: Klebsiella pneumoniae (K. pneumoniae) rapidly acquires resistance to antibiotics, posing a major challenge to effective clinical management. In contrast, the discovery and development of new antibiotics are time-consuming, resource-intensive, and costly, highlighting the need for alternative therapeutic strategies. One promising approach to identify novel and/or alternative therapeutic strategies is to exploit collateral sensitivity, whereby the evolution of resistance to one antibiotic results in increased susceptibility to another. In this study, we established adaptive laboratory evolution models by exposing K. pneumoniae (ATCC and clinical isolates) to sequentially increasing concentrations of colistin and meropenem, as well as by subjecting colistin-resistant (1× MIC) K. pneumoniae to stepwise meropenem exposure over a cumulative period of approximately 250 days. The evolved strains were subsequently characterized using label-free LC-MS/MS proteomic profiling to investigate the emergence of antibiotic-resistance and collateral responses between colistin and meropenem at both phenotypic and molecular levels. In total, 3,126 proteins were identified across all experimental conditions.

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Sample Preparation: The panels of developed resistant K. pneumoniae cells representing parent (each for colistin and meropenem) were cultured by adding the respective antibiotic concentrations and harvested in the mid-exponential phase of culture (after 4h. of incubation). The cells were washed twice with cold phosphate buffered saline, and the pellet was stored at -80? until use. In-sol trypsin digestion was performed by denaturing 50 µg of protein with 8M urea at 1 mg/mL concentration. 10mM DTT was used for reduction for up to 1h at 37?, followed by alkylation with 20mM IAA in the dark. 50mM ammonium bicarbonate was added to the sample mixture to dilute the concentration to 2M. Proteins were digested with trypsin at a ratio of 1:20 overnight at 37?. Following digestion, the resulting peptide mixture was acidified with formic acid,

desalted, and vacuum-concentrated.

Peptide Separation: Orbitrap Exploris™ 480 system with a FAIMS Pro™ interface. FlexMix calibration solution (Pierce) was used to tune the instrument before data acquisition. The sample data were acquired using a nanospray ionization ion source with a voltage 2000 V, ion transfer tube temperature 280oC, FAIMS voltage of-50oC, RF lens 40%, automatic gain control 300%, maximum injection time in automode, and one microscan

Protein Characterization: Proteins in the samples was performed via database searching against K. pneumoniae, downloaded from the UniProt database using Proteome discoverer software with the Chimerys algorithm with inference rescoring. The search parameters were as follows: enzyme trypsin, maximum missed cleavage 2, mass tolerance 10 ppm, static modification Cys carboxamidomethyl, dynamic modification Met oxidation, target/decoy strategy using percolator, target FDR 1%

Experiment Type: Bottom-up

Species: Klebsiella pneumoniae No Data

Tissue: N/A No Data

Cell Type: Bacteria No Data

Disease: N/A No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications:

PubMed ID: