IPD Project Details

Project ID: IPD3489

Project Title: Proteomic profiling of Klebsiella pneumoniae resistant against short-duration exposure (SDE) dose of colistin and meropenem.

Description: Short-duration exposure (SDE) of antibiotics is among the major drivers of the emergence of antimicrobial resistance (AMR) among bacterial pathogens. Klebsiella pneumoniae is a priority-1 (critical) bacterial pathogen rapidly acquiring MDR/XDR traits, even against next-generation antibiotics. Since meropenem (latest generation) and colistin (last-resort) antibiotics for treating K. pneumoniae infections and new antibiotic development is resource-intensive, understanding how resistance emerges following SDE is crucial for devising strategies to preserve antibiotics for long-term/future use. With this aim, we developed colistin-resistant and meropenem-resistant K. pneumoniae using SDE of antibiotics (1x, 2x, and 4x MICs) and proteome profiled. A total of 1379 proteins were identified using LC-MS.

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Sample Preparation: Model resistant K. pneumoniae cells representing parent strain 0x (WT/control), resistant at 1x MIC, 2x MIC and 4x MIC for colistin were cultured by adding respective adapted antibiotics concentration and harvested in the mid-exponential phase of culture (post 4 hrs. of incubation). Cold PBS was used to wash the cells twice, and the pellet were stored at -80? until used. 50 µg protein was denatured by 8M urea at 1mg/mL concentration. 10mM DTT was used for reduction for up to 1 hrs. at 37? followed by alkylation with 20mM IAA in dark. 50mM ammonium bicarbonate was added to the sample mixture to dilute the urea concentration to 2M. Proteins were digested with trypsin at 1:20 for overnight at 37?. Following digestion, the resulting peptide mixture was then acidified using formic acid, desalted, and vacuum concentrated.

Peptide Separation: LC-MS/MS was done using a Vanquish™ Neo UHPLC System, connected on-line with a Orbitrap Exploris™ 480 Mass Spectrometer (Thermo Scientific). The trypsin digested samples were desalted offline by C18 column prior to loading them on a Trap (PepMap™ Neo Trap Cartridge 100 A, 5 ?m C18 0.3 mm x 5 mm) and

analytical nano column (Easy-Spray™ PepMap™ Neo 2 ?m C18 75 ?m x 150 mm). The mobile phase for HPLC was as follow: water/formic acid (100/0.1%) and acetonitrile/formic acid (80/0.1%). Sample (500 ng) was injected into the column with a flow rate of 300 nl/min. Analytical separation was established by the following gradient conditions: initial 6% B for 2 min, followed by a linear gradient to 31% B in 58 min, followed by another linear gradient to 44% B in 30 min. Following the peptide elution window, the gradient was increased to 100% B in 1 min and held for 9 min.

Protein Characterization: Data-dependent acquisition experiment was performed on a Orbitrap Exploris™ 480 system having a FAIMS Pro™ Interface. FlexMix calibration solution (Pierce) was used to tune the instrument before data acquisition. The sample data was acquired using a nano-spray ionization ion source with a voltage 2000 V, ion transfer tube temperature 280?, FAIMS voltage -50?, RF lens 40 %, automatic gain control 300%, maximum injection time in auto mode and 1 microscan. The MS was operated at a resolution of 60,000 FWHM and a mass range of m/z 350 - 1200. A total of 30 survey scans were acquired at 15,000 resolution and 28% HCD collision energy, if exceeding an intensity threshold of 5.0e3 with a 2+ to 6+ charge states. Dynamic exclusion duration was set for 45s.

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Bacteria No Data

Disease: Data in disease_details No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications:

PubMed ID: