P0770
Paper Poster Session
The complexity of antibacterial resistance mechanisms

Quantitative proteome analysis of an Escherichia coli exposed to tetracycline reveals multiple affected cytoplasmic metabolic processes

Daniela Jones-Dias*[1], Ana Sofia Carvalho[2], Inês Moura[1], Brian Dill[3], Henrik Molina[3], Manuela Caniça[1], Rune Matthiesen[2]

[1]*National Institute of Health Dr. Ricardo Jorge, Department of Infectious Diseases, Lisbon, Portugal*

[2]*National Institute of Health Dr. Ricardo Jorge, Computational and Experimental Biology Group, Lisbon, Portugal*

[3]*The Rockefeller University, Proteomics Resource Center, New York, United States*

Background: Many studies have already focused on the evaluation of variations in protein expression caused by antibiotic exposure in tetracycline resistant microorganisms. However, little is known about the metabolic response of genetically resistant bacterial populations to antibiotic exposure. In this study we used a liquid chromatography-mass spectrometry-based (LC-MS/MS) proteomics to evaluate the global cytoplasmic metabolic changes of a resistant *Escherichia coli* isolate, when challenged with tetracycline.

Material/methods: IsolateEcAamb278 was recovered from a soil sample, used for the intensive farming of tomato plants and showed nonsusceptibility to all categories of β-lactams except carbapanems and cefoxitin, and tetracycline. Previous genomic characterization revealed the presence of TEM-1, CTX-M-1, Sul2,TetA and TetB resistance determinants. Single colonies of *E. coli*EcAamb278 were used to extract crude cytoplasmic protein fraction using non detergent cell lysis buffer. The proteomic profile of *E. coli* EcAmb278 was determined by label-free LC-MS/MS upon tetracycline stress in a comparative study, using as referencethe proteome of the same strain non-exposed to antibiotics. The samples were analyzed by nano LC-MS/MS, using a Q-Exactive mass spectrometer. All data was searched with VEMS. Proteins were quantified by spectral counting and mziXIC, followed by iBAQ estimation.

Results: The complete proteome yielded about 1484 proteins, using a 1% FDR as cut off. The comparison of the proteome profiles of the two *E. coli* EcAamb278 samples pointed to several proteins with altered expression under tetracycline stress conditions. The twelve most significant (FDR-corrected $p<0.05$) proteins differentially regulated by more than two-fold were involved in DNA metabolism, transcription, virulence, intracellular trafficking and secretion, biosynthesis of vitamins, other processes, and unknown functions. For instance, (1) acyl-CoA thioester hydrolase, involved in biosynthesis of coenzyme A; (2) bacterial NAD^+-dependent DNA ligase, plays a critical role in DNA replication, recombination and repair in all living organisms; and (3) adhesion protein FimH, which confirmed the pathogenicity of this environmental-borne *E. coli*. Overall, several other detected proteins were associated with the process of antibiotic resistance, virulence and acquisition and transfer of foreign DNA (AcrA, TolC, MdtE, Omps, TnsE, Colicin peptides, among others).

Conclusions: These results indicate that *E. coli* responses to tetracycline are related to protein translation as well as metabolic regulation. We observed differentially regulation of several metabolic proteins though not belonging to the canonical antibiotic resistance pathway, suggesting an integrated

metabolic bacteria response to antibiotic exposure, which can potentially be explored for drug development.