**Materials and Methods**

**Preparation of *Escherichia coli* proteins for mass spectrometry**

The bacterial strain *Escherichia coli* NCTC 13400, containing the MDR conjugative plasmid pEK499, was used in all experiments. The pEK499 plasmid was 117,536 bp in length and belongs to incompatibility group F as represented a fusion of two replicons of types FII and FIA (2). *Escherichia coli* (NCTC 13400) containing the MDR plasmid pEK499 was exposed to antimicrobials for which the bacteria displayed a resistance phenotype (ampicillin 64mg/L, cefotaxime 256 mg/L) and those, which there was no resistance phenotype (imipenem 0.06 mg/L, ciprofloxacin 0.06 mg/L) (2). The control comprised the *E. coli* with pEK499 grown without antimicrobial. All strains were grown separately in Luria-Bertani (LB) at 37 ⁰C with shaking at 200 rpm. All experiments were performed in biological triplicates. Cells were harvested by centrifugation at 3000 rpm for 15 minutes. The cell pellet was resuspended in ammonium bicarbonate (1 ml, 50 Mm, pH 7.8) and sonicated on ice in 10 second bursts five times. The lysate was subjected to centrifugation at 13,000 rpm to collect the cellular debris. The supernatant was quantified using the QubitTM quantification system (Invitrogen), following the manufacturer’s instructions. The protein sample was reduced by adding 5 μl 0.2 M dithiothreitol (DTT) and incubated at 95°C for 10 minutes, followed by alkylation with 0.55 M iodoacetamide (4 µl) at room temperature, in the dark for 45 minutes. Alkylation was stopped by adding DTT (20 µl, 0.2 M) and incubation for 45 minutes at 25 °C. Sequence Grade Trypsin (Promega) (0.5 µg/µl) was added to the proteins and incubated at 37°C for 18 hours. The digested protein sample was brought to dryness using a Speedyvac concentrator (Thermo Scientific Savant DNA120). Samples were purified for mass spectrometry using C18 Spin Columns (Pierce), following the manufacturer’s instructions. The eluted peptides were dried in a SpeedyVac concentrator (Thermo Scientific Savant DNA120) and resuspended in 2% *v/v* acetonitrile and 0.05% *v/v* Trifluoroacetic acid (TFA) to give a final peptide concentration of 1 µg/µl. The samples were sonicated for five minutes to aid peptide resuspension, followed by centrifugation for five minutes at 13,000 rpm*.* The supernatant was removed and used for mass spectrometry. Three independent biological replicates for each group were analysed.

**Mass Spectrometry: LC/MS Xcalibur Instrument parameters for proteomic data acquisition**

Digested proteins (1 µg) isolated from the replicates for each *E. coli* sample were loaded onto a QExactive (ThermoFisher Scientific) high-resolution accurate mass spectrometer connected to a Dionex Ultimate 3000 (RSLCnano) chromatography system. Peptides were separated by an increasing acetonitrile gradient on a 50 cm EASY-Spray PepMap C18 column with 75 µm diameter (2 µm particle size), using a 180 minute reverse phase gradient at a flow rate of 300 nL/mi -1 n. All data were acquired over 141 minutes, with the mass spectrometer operating in an automatic dependent switching mode. A full MS scan at 140,000 resolution and a range of 300 – 1700 *m/z*, was followed by an MS/MS scan at 17,500 resolution, with a range of 200-2000 *m/z* to select the 15 most intense ions prior to MS/MS.

Quantitative analysis (protein quantification and LFQ normalization of the MS/MS data) of the *E. coli*  proteome arising from exposure to the different antimicrobials, was performed using MaxQuant version 1.6.3.3 (<http://www.maxquant.org>) following the general procedures and settings outlined in Hubner et al., 2010 (6). The Andromeda search algorithm incorporated in the MaxQuant software was used to correlate MS/MS data against the Uniprot-SWISS-PROT database for *E. coli* K12(4319 entries)and the *E. coli* strain plasmid pEK499 (141 entries). The following search parameters were used: first search peptide tolerance of 20 ppm, second search peptide tolerance 4.5 ppm with cysteine carbamidomethylation as a fixed modification and N-acetylation of protein and oxidation of methionine as variable modifications and a maximum of two missed cleavage sites allowed. False discovery rate (FDR) was set to 1 % for both peptides and proteins, and the FDR was estimated following searches against a target-decoy database. Peptides with a minimum length of seven amino acid length were considered for identification and proteins were only considered identified when observed in three replicates of one sample group.

**Data Analysis of the proteome**

Perseus v.1.5.5.3 (www.maxquant.org/) was used for data analysis, processing and visualisation. Normalised LFQ intensity values were used as the quantitative measurement of protein abundance for subsequent analysis. The data matrix was first filtered for the removal of contaminants and peptides identified by site. LFQ intensity values were log2 transformed and each sample was assigned to its corresponding group. Proteins not found in all three replicates in at least one group were omitted from the analysis. A data-imputation step was conducted to replace missing values with values that simulate signals of low abundant proteins chosen randomly from a distribution specified by a downshift of 1.8 times the mean standard deviation (SD) of all measured values and a width of 0.3 times this SD.

Normalised intensity values were used for a principal component analysis (PCA). Exclusively expressed proteins (those that were uniquely expressed or completely absent in one group) were identified from the pre-imputation dataset (Supplemental dataset 1) and included in subsequent post-imputation analyses (Supplemental dataset 2). To visualise differences between two samples, pairwise Student’s t-tests were performed for all using a cut-off of p<0.05 on the post-imputated dataset. Volcano plots were generated in Perseus by plotting negative log p-values on the y-axis and log2 fold-change values on the x-axis for each pairwise comparison. The ‘categories’ function in Perseus was utilized to highlight and visualise the distribution of various pathways and processes on selected volcano plots. Statistically significant (ANOVA, p<0.05) proteins were chosen for further analysis. Gene ontology (GO) mapping was also performed in Perseus using the UniProt gene ID for all identified proteins to query the Perseus annotation file (downloaded September 2018) and extract terms for gene ontology biological process (GOBP), gene ontology cellular component (GOCC), gene ontology molecular function (GOMF) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) name. Enrichment analysis was performed in Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), using a high confidence setting (0.700), and hiding disconnected nodes in the network. Statistically significant protein names arising from pairwise t-tests were inputted into the STRING database to identify interactions occurring between proteins that were increased or decreased in relative abundance between a treatment and the control. The MS proteomics data and MaxQuant search output files have been deposited to the ProteomeXchange Consortium (7) via the PRIDE partner repository with the dataset identifier PXD027164.