Exploring the effects of bacterial infection and antibiotic or faecal microbiota transplantation treatments on the mouse gut microbiome

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Overview

- In this work mice were infected by the bacterial pathogen *Clostridioides difficile* and treated with either the antibiotic metronidazole or faecal microbiota transplantation (FMT) as a model to compare effects of bacterial infection and treatments on the gut microbiome.
- The analysis of FMT treated mice exhibited gut microbiome metabolic profiles broadly similar to uninfected controls; following infection or treatment with the antibiotic metabolite profiles differed from controls, but not in the FMT treated group.

1. Introduction

The gut microbiome is a key driver in metabolic signalling influencing immune responses and protecting against pathogens. Changes to gut microbial communities caused by a disease state influence host physiology via several pathological mechanisms. Likewise, treatments of bacterial infections have their own mechanistic effects on the gut microbiome. In this study, *Clostridioides difficile* infection (CDI) has been selected as a model to study the impact of a bacterial pathogen on the gut microbiome as well as the effects of an antibiotic (metronidazole) versus faecal microbiota transplantation (FMT) used in treatment. Metabolic profiling of murine caecum tissue and faecal gut content was performed using high resolution LC-MS/MS with targeted and untargeted data processing.

2. Materials and Methods

Thirteen week old male C57BL/6 mice were treated for four days with an antibiotic cocktail and administered an intraperitoneal injection of clindamycin 10 mg kg^{-1} on day six. One group was left uninfected, while other groups received non-toxinogenic (TCDA-, TCDB-) CDI on day 7. Of the three groups infected, one remained untreated while the other groups received a 10-day course of either metronidazole or 10% faecal water in drinking water, prepared from faecal samples of uninfected controls. Faecal gut content and caecum tissues collected post-mortem were extracted in methanol:isopropanol:water 1:1:2 v/v/v in a ratio of tissue:solvent 3:1 then in methanol:MTBE 1:3 v/v.

- **Reverse Phase LC Separation (cycle time 35 mins)**
  - Samples reconstituted in methanol; pooled QC analysed throughout batch
  - Acquity C18 BEH (2.1x100mm 1.7µm); 50ºC, flow rate 0.4 mL/min
  - Binary gradient: water + 0.1% formic acid; acetonitrile + 0.1% formic acid
- **HILIC LC Separation (cycle time 18 mins)**
  - Pooled QC reconstituted in 90% acetonitrile analysed to support metabolite ID
  - Shim-pack Velox HILIC (2.1x100mm 2.7µm); 40ºC, flow rate 0.3 mL/min
  - Binary gradient; water + 10mM ammonium formate 0.1% formic acid, and acetonitrile:water+ 10mM ammonium formate 0.1% formic acid [92:8]
- **Mass Spectrometry Detection** QTOF LCMS-9030; external mass calibration; ESI+/-
  - MS mass scan m/z 65-1010; 100 msecs
  - DIA-MS/MS mass scans m/z 40-1000; 33 msecs for each precursor isolation window; isolation width 35 Da; collision energy spread 5-55V; 27 mass scan events. Scan cycle time 0.99 second (28 mass scans in total).
- **Data processing**
  - Untargeted processing used MS-Dial and feature annotation by comparison to online repositories such as MassBank, mzCloud and LipidMaps.
  - Targeted processing used LabSolutions Insight with identification against in house MS/MS libraries of authentic standards where available.

3. Results

3.1 Untargeted analysis of infection and treatment

Early elution feature extraction
Retention times for different metabolite classes
0-3 mins; nucleotides, amino acids, purines, pyrimidines and associated derivatives.
2-6 mins; bile acids
0-8 mins; carnitines

Reverse phase method developed primarily for lipid signals
Retention times for different metabolite classes
6-10 mins; LPLs
7-16 mins; Fatty acids and related compounds 8-12 mins; MGs
22 mins; cholesterol
22-30 mins; PLs, SLs and DGs

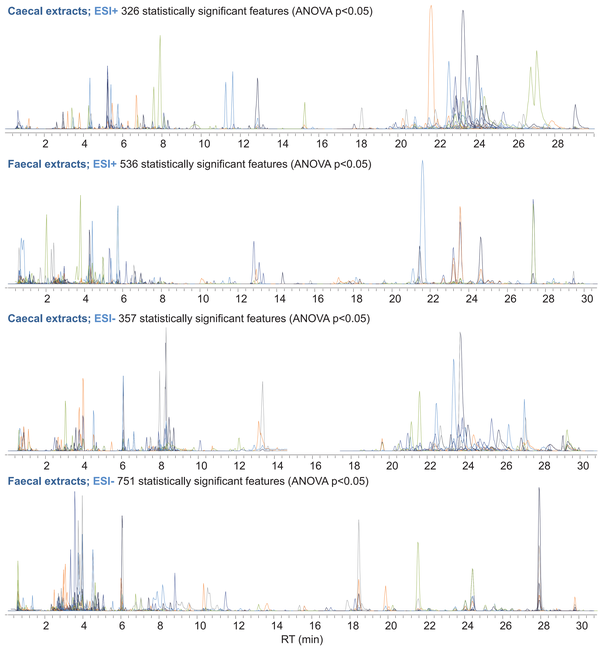


Figure 1. Mass chromatograms of ion signals found to be statistically different between groups by ANOVA (p<0.05) in each dataset: caecal or faecal extract analysed in ESI+ or ESI -.

Data analysis in MetaboAnalyst post processing in MS-Dial

- Ion signals present in less than 80% of QCs or at RSD>20% filtered out; ion signals present in less than 25% of sample filtered out; known adducts filtered out.
- 6240 and 5408 features in ESI+ and ESI- datasets for caecal extracts considered for statistical analysis; 7869 and 6558 features in ESI+ and ESI- datasets for faecal extracts.
- A large proportion of the significant features could not be positively identified to MSI levels 1, 2 or 3.

3.2 Targeted effects of different treatments

Figure 2. Box and whisker plots highlighting differentially expressed metabolites in caecal and faecal extracts infected with CDI and treated with metronidazole or FMT.

Biological results

- In caecal extracts LPE 16:1 and LPE 18:1 (both sn-2 isoforms), malate, proline and creatine were all significantly increased following the antibiotic metronidazole treatment.
- Taurocholate, creatinine and cysteate significantly increased in faecal gut content following infection but reverted to the concentration of the uninfected controls with FMT treatment.
- The bacterial pathogen infection lowered the concentration of N-acyl taurines (N-linoleoyl taurine, N-arachidonoyl taurine, N-oleoyl taurine) in caecal tissue but this effect was reversed to some extent following FMT.

Data analysis

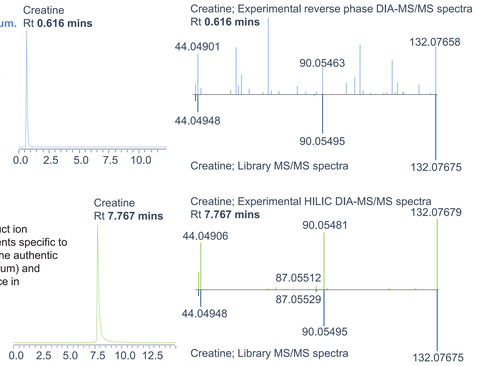
- LabSolutions Insight was used for feature finding and MetaboAnalyst for statistical analysis.
- Metabolites were identified with MSI level 1 and 2 confidence using an in-house library of MS/MS product ion spectra acquired using authentic reference material where available. The library included accurate mass spectra for lipid distributions (LPC's, LPEs, MGs, fatty acids, fatty acid conjugates and fatty amides) and polar metabolites (nucleotides, amino acids, purines and pyrimidines).

3.3 Increasing confidence in metabolite identification

To increase reporting confidence for highly polar metabolites (eluting between 0-2 minutes by the reverse phase LC-MS/MS method), the same samples were analysed by an optimised HILIC method. Metabolite features were identified using an in-house library acquired using the HILIC LC-MS/MS method;

- MS/MS data acquired using authentic standards with a fragmentation energy of 5-55V.
- All product ion spectra corrected to theoretical accurate mass values using Insight Assign application.
- MS/MS spectra acquired using targeted MS/MS and a precursor ion isolation width of unit mass. Spectra were acquired positive and negative ion modes
- Retention time mapped to a Shim-pack Velox HILIC LC separation.

Reverse Phase Method
Chimeric MS/MS spectrum.
Identifying highly polar metabolites with higher confidence may require a complementary approach such as HILIC to remove overlapping product ion spectra.



HILIC method
MS/MS spectrum.
In this example, the product ion spectrum contains fragments specific to creatinine (confirmed by the authentic reference standard spectrum) and supports further confidence in compound identification.

Figure 3. Extracted ion chromatograms for creatine detected by reverse phase (above) and HILIC (below). DIA-MS/MS spectra from both were positively identified as creatine by reference material reflected in each plot.

4. Conclusions

- Metabolite profiling in mice infected with the bacterial pathogen and treated with the antibiotic metronidazole showed significant changes in the analysis of caecal tissue and faecal gut content.
- The analysis of FMT treated mice exhibited gut microbiome metabolic profiles broadly similar to uninfected controls
- LC-MS/MS data was acquired using a DIA which is applicable to both targeted and untargeted workflows for data analysis.
- Targeted workflows benefit from rapid and reliable identification of metabolites with the highest reporting confidence (MSI level 1 and 2), while untargeted workflows allow a wider exploration of the differences between phenotypes, but identification is more challenging, particularly in less well-defined matrices such as faeces, with many significant features being unidentifiable or identifiable to MSI levels 2, 3 or 4.