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# P 176 EFFECTS OF PLANT-BASED DIET ON GUT MICROBIOME COMPOSITION, HOST METABOLOME AND MICROBIOME RESILIENCE IN THE CONTEXT OF Clostridioides difficile INFECTION

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### **Background and Objectives:**

Gut microbiome resilience is the restorative capacity of the microbiome following a perturbative event (e.g. antibiotics or unhealthy diet). Healthy diets (e.g. Mediterranean) are inversely correlated with chronic disease risk, being high in fruit and vegetables, rich in fibre, low in simple sugars and saturated fat, in contrast to the westernised diet, high in saturated fat and refined foods. Impairment of the gut microbiome allows infection by opportunistic pathogens (pathobionts) e.g. *C. difficile*, a major cause of healthcare-associated infection. The colonic milieu reflects metabolic outputs of specific dietary patterns (e.g. (poly)phenols and SCFA), faecal water (FW) is therefore of physiological relevance as it is the aqueous phase of faeces. The effects of westernized (W) and plant-based (PB) diets on host metabolome and microbiome composition were investigated. Subsequently FW derived from both dietary patterns was assessed to determine its impact on *C. difficile* biology.

### Methods:

A single-blind, randomised, controlled two-way crossover dietary intervention study (Clinical Trials.gov NCT05231317) was conducted in 11 participants (BMI > 18, aged 18-70, free-living, non-smokers) which consumed two diets: PB diet (polyphenol rich) and W diet (high saturated fat, high in processed foods) for 14 consecutive days, separated by a 7-weeks washout period. Participants were sampled (blood, urine, faeces) pre and post intervention periods. Metabolites concentrations were evaluated *via* NMR in plasma, urine, and faecal water. Plasmatic bile acids concentrations were evaluated with UHPLC-MS/MS and (poly)phenols in urine *via* UHPLC-HRMS. Microbiota composition was analysed via 16S rRNA amplicon sequencing. The best responder to the PB diet was selected and FW growth media (day 1 & 14) prepared for both intervention periods. *C. difficile* 630 was then cultured with these FW enriched growth media and cell pellets harvested (mid-exponential log growth phase), RNA extracted, and transcriptomic analysis (RNA-seq) was performed on *C. difficile* 630 to determine the differential effect of diet on *C. difficile* biology.

#### Results

PB diet displayed significant reduction in plasmatic total and primary bile acids compared to the W diet. PB diet significantly increased urinary (poly)phenolic compounds: m-coumaric acid, 3-(3'-Methoxy-4'-hydroxyphenyl)propanoic acid, 3-(4'-Methoxy-3'-hydroxyphenyl) propanoic acid, 4'-hydroxymandelic acid). Urinary creatine, associated with meat consumption decreased significantly. PB diet had limited impact on the gut microbiota with significant reduction in *Subdoligranulum* genus only. Preliminary analysis of *C. difficile* transcriptomes from bacterial cells cultured in FW from PB & W diets (day 14), revealed significant global changes in gene expression involving transport binding proteins and lipoproteins (12.9% of total), metabolism of amino acids (7.6%), glycolytic pathways (4.2%) and sporulation genes transcription (2.4%). PB diet had a differential effect on implied *C. difficile* pathogenicity (sporulation) compared to the W diet, significant decreases (p<0.001) were observed in the master regulator *spo0A*, genes involved in stage II sporulation (e.g *spoIIGA*, *spoIIE*), stage V (e.g. *spoVAD*), while the expression of genes encoding spore coat proteins (CotA, CotE and stage V proteins) were significantly increased (p<0.001).

## **Conclusions:**

The data suggest that diets rich in fruits and vegetables can beneficially alter the pathobiology of the opportunistic human pathogen *C. difficile*.