CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent Clostridioides difficile infection
Authors	Mullish, Benjamin H.;McDonald, Julie A. K.;Pechlivanis, Alexandros;Allegretti, Jessica R.;Kao, Dina;Barker, Grace F.;Kapila, Diya;Petrof, Elaine O.;Joyce, Susan A.;Gahan, Cormac G.;Glegola-Madejska, Izabela;Williams, Horace R. T.;Holmes, Elaine;Clarke, Thomas B.;Thursz, Mark R.;Marchesi, Julian R.
Publication date	2019-02-11
Original Citation	Mullish, B.H., McDonald, J.A., Pechlivanis, A., Allegretti, J.R., Kao, D., Barker, G.F., Kapila, D., Petrof, E.O., Joyce, S.A., Gahan, C.G.M., Glegola-Madejska, I., Williams, H.R.T., Holmes, E., Clarke, T.B., Thursz, M.R. and Marchesi, J.R. (2019) 'Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent Clostridioides difficile infection'. Gut, 68, pp.1791-1800. doi:10.1136/gutjnl-2018-317842
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://gut.bmj.com/content/early/2019/02/11/gutjnl-2018-317842 - 10.1136/gutjnl-2018-317842
Rights	© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY. Published by BMJ. This is an open access article distributed in accordance with the Creative Commons Attribution 4.0 Unported (CC BY 4.0) license, which permits others to copy, redistribute, remix, transform and build upon this work for any purpose, provided the original work is properly cited, a link to the licence is given, and indication of whether changes were made. See: https://creativecommons.org/licenses/by/4.0/. - https://creativecommons.org/licenses/by/4.0/
Download date	2026-10-30 15:02:04
Item downloaded from	https://hdl.handle.net/10468/9227



University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

OPEN ACCESS

ORIGINAL ARTICLE

Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent *Clostridioides difficile* infection

Benjamin H Mullish,[1] Julie A K McDonald,[1] Alexandros Pechlivanis,[1] Jessica R Allegretti,[2,3] Dina Kao,[4] Grace F Barker,[1] Diya Kapila,[1] Elaine O Petrof,[5] Susan A Joyce,[6,7] Cormac G M Gahan,[6,8] Izabela Glegola-Madejska,[9] Horace R T Williams,[1] Elaine Holmes,[1] Thomas B Clarke,[9] Mark R Thursz,[1] Julian R Marchesi[1,10]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2018-317842).

For numbered affiliations see end of article.

Correspondence to
Professor Julian R Marchesi, Division of Integrative Systems Medicine and Digestive Disease, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London SW7 2AZ, UK; j.marchesi@imperial.ac.uk

Received 31 October 2018
Revised 26 December 2018
Accepted 15 January 2019

Check for updates

© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY. Published by BMJ.

To cite: Mullish BH, McDonald JAK, Pechlivanis A, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2018-317842

ABSTRACT

Objective Faecal microbiota transplant (FMT) effectively treats recurrent *Clostridioides difficile* infection (rCDI), but its mechanisms of action remain poorly defined. Certain bile acids affect *C. difficile* germination or vegetative growth. We hypothesised that loss of gut microbiota-derived bile salt hydrolases (BSHs) predisposes to CDI by perturbing gut bile metabolism, and that BSH restitution is a key mediator of FMT's efficacy in treating the condition.

Design Using stool collected from patients and donors pre-FMT/post-FMT for rCDI, we performed 16S rRNA gene sequencing, ultra performance liquid chromatography mass spectrometry (UPLC-MS) bile acid profiling, BSH activity measurement, and qPCR of *bsh*/*bai*CD genes involved in bile metabolism. Human data were validated in *C. difficile* batch cultures and a C57BL/6 mouse model of rCDI.

Results From metataxonomics, pre-FMT stool demonstrated a reduced proportion of BSH-producing bacterial species compared with donors/post-FMT. Pre-FMT stool was enriched in taurocholic acid (TCA, a potent *C. difficile* germinant); TCA levels negatively correlated with key bacterial genera containing BSH-producing organisms. Post-FMT samples demonstrated recovered BSH activity and *bsh*/*bai*CD gene copy number compared with pretreatment ($p<0.05$). In batch cultures, supernatant from engineered *bsh*-expressing *E. coli* and naturally BSH-producing organisms (*Bacteroides ovatus, Collinsella aerofaciens, Bacteroides vulgatus* and *Blautia obeum*) reduced TCA-mediated *C. difficile* germination relative to culture supernatant of wild-type (BSH-negative) *E. coli*. *C. difficile* total viable counts were ~70% reduced in an rCDI mouse model after administration of *E. coli* expressing highly active BSH relative to mice administered BSH-negative *E. coli* ($p<0.05$).

Conclusion Restoration of gut BSH functionality contributes to the efficacy of FMT in treating rCDI.

Significance of this study

What is already known on this subject?

- Faecal microbiota transplant (FMT) effectively treats recurrent *Clostridioides difficile* infection (rCDI), but has drawbacks associated with its use. Furthermore, its mechanisms of action remain poorly defined.
- There is an association between rCDI and altered gut bile acid profiles, but the significance of this remains unclear.

What are the new findings?

- Patients successfully treated for rCDI by FMT showed a rapid and sustained enrichment in gut microbiota members which produce a bile-metabolising enzyme, bile salt hydrolase (BSH).
- Relative abundance of gut BSH-producing organisms correlates negatively with stool levels of taurocholic acid (TCA), a potent trigger for *C. difficile* germination. TCA is hydrolysed by BSH to cholic acid; this is subsequently metabolised into deoxycholic acid, which potently inhibits the growth of *C. difficile*.
- In batch cultures, BSH-producing microorganisms completely reversed TCA-mediated germination of *C. difficile*.
- BSH-producing microbes significantly reduced faecal counts of *C. difficile* after administration to an rCDI mouse model compared with administration of a BSH-negative microbes.

How might it impact on clinical practice in the foreseeable future?

- Restored gut BSH functionality plays a central role in the efficacy of FMT in treating CDI.
- Targeted restoration of this function within the gut (eg, BSH-producing microorganisms, purified BSH enzyme) represents a novel therapeutic approach to treating rCDI which avoids the limitations associated with FMT.
- BSH supplementation merits further evaluation for its potential role as a therapy for human patients with rCDI.

INTRODUCTION

Clostridioides difficile (previously named *Clostridium difficile*[1]) infection (CDI) is the major global cause of nosocomial GI infection, with incidence

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

rates increasing worldwide.[2 3] One therapeutic strategy for recurrent CDI (rCDI) that has come to prominence is faecal microbiota transplant (FMT). FMT has been demonstrated to be a highly effective therapy for rCDI, with cure rates of >80% in randomised trials,[4] and up to >90% in case series where it was administered colonoscopically.[5] However, FMT is not without drawbacks, including its unpalatability, the theoretical risk of infection transmission and its regulatory complexity.[6]

Understanding the mechanisms underlying the efficacy of FMT in treating CDI may allow formulation of novel, more targeted, anti-CDI therapeutics. In a pilot study, sterile faecal filtrate was shown to be an effective treatment for rCDI in five patients,[7] consistent with bacterially derived proteins, gut metabolites, bacteriophages or other filtrate components mediating the efficacy of FMT in treating this condition, as opposed to intact microorganisms. While the possible contribution of such mediators to FMT are starting to be elucidated,[8–10] they remain incompletely defined.

One particular area of interest concerns the interaction between the gut microbiota and host bile acid metabolism in rCDI. In vitro, certain bile acids differentially affect the ability of *C. difficile* to undergo germination and vegetative growth. In particular, the conjugated primary bile acid taurocholic acid (TCA) is a potent trigger of *C. difficile* germination (with glycine as co-germinant),[11] while the secondary bile acid deoxycholic acid (DCA) markedly inhibits vegetative growth.[11 12] The transition from conjugated primary bile acids to secondary bile acids in vivo principally involves two enzymatic steps, with both enzymes produced by microbes but not mammals (see online supplementary figure 1). The first step is undertaken by bile salt hydrolases (BSHs), which deconjugate the taurine and glycine groups via a hydrolysis reaction, and consequently reform the unconjugated primary bile acids cholate and chenodeoxycholate. BSHs are widely distributed throughout most major bacterial divisions and archaea species of the gut microbiota, and at least eight different *bsh* genes exist[13] (see online supplementary figure 2). The second enzymatic step is 7-α-dehydroxylation, whereby unconjugated primary bile acids are converted to secondary bile acids, including deoxycholate and lithocholate.

At present, there are only limited data exploring the possible contribution of BSHs to CDI vulnerability,[14] and none investigating the effect of FMT on BSH functionality. Given the key contribution of BSH's substrate, TCA, to the germination of *C. difficile*, this is a clear potential mechanistic explanation for the efficacy of FMT. As such, using a work flow including human samples, batch cultures and mouse models, we investigated the hypothesis that patients with rCDI are deficient in gut microbiota members which produce BSH, with the consequent enrichment in TCA (promoting *C. difficile* germination) and loss of DCA (permitting vegetative growth) contributing to ongoing disease. We further hypothesised that successful FMT recolonises the gut microbiota with BSH-producing organisms, contributes to the restoration of the normal bile acid *milieu* of the gut, and consequently removes key triggers for *C. difficile* germination and vegetative growth.

MATERIALS AND METHODS

Study participants and FMT protocols

For the main human data set, stool samples were collected from participants with rCDI (26 participants; samples collected pre- FMT and at 8–12 weeks post-FMT) and their FMT donors (17 participants). rCDI was diagnosed on a combination of clinical and laboratory criteria (see online supplementary methods 1.1). Patient characteristics are described in online supplementary table 1; none of the included patients had IBD. For validation of initial findings, stool samples were also analysed for patients with rCDI from a Canadian randomised controlled trial investigating capsulised versus colonoscopic FMT as rCDI treatment (18 participants; samples collected pre-FMT and at 1, 4 and 12 weeks post-FMT), together with donors (five participants).[15] FMT slurry was also collected from these donors.

Donor inclusion/exclusion criteria, screening and testing followed previously described recommendations.[16] FMT protocols used are detailed in online supplementary methods 1.1.

DNA extraction and 16S rRNA gene sequencing

DNA was extracted from 250 mg of stool using a previously described protocol.[17] 16S rRNA gene qPCR data were used to determine total bacterial biomass within each sample (see online supplementary methods 1.2). Sample libraries were prepared following Illumina's 16S Metagenomic Sequencing Library Preparation Protocol[18] with several modifications.[17] The V1-V2 regions of the 16S rRNA gene were amplified using previously reported primers.[17] The methodology for metataxonomic analysis is described in online supplementary methods 1.3. We also predicted the bile metabolising ability of microbial communities using a metagenomic inferential tool, Piphillin[19] (see online supplementary methods 1.4).

UPLC-MS profiling of faecal bile acids

The protocol used for initial sample processing[17] and for analysis[20] was as previously described. Methodology for preprocessing and analysis of ultra performance liquid chromatography mass spectrometry (UPLC-MS) bile acid data is described in online supplementary methods 1.5.1. Integration of metataxonomic and UPLC-MS bile profiling data is described in online supplementary methods 1.6

Abundance and activity of bile metabolising enzymes

qPCR was performed using extracted DNA to quantify gene abundance for (1) specified groups of *bsh* (using degenerate primer sets previously reported)[17] and (2) *bai*CD (using previously described primers[21]) (see online supplementary methods 1.7). The BSH activity assay was an adaptation of the conventional precipitation-based assay,[22–24] using a previously described technique.[17]

C. difficile germination batch cultures

These were performed via adaptation of a previously described protocol.[11] *C. difficile* spore preparation and enumeration is described in online supplementary methods 1.8. A range of different bacterial species established to produce BSH from different BSH groups (and *Clostridium scindens*, as a known 7-α-dehydroxylase-producer) were incubated in sBHI (brain heart infusion broth (Sigma-Aldrich), with 5 mg/mL yeast extract (Sigma-Aldrich), and 0.1% w/v L-cysteine (Sigma-Aldrich)), with or without 1% w/v TCA added (see online supplementary methods 1.7). This list also included wild-type *E. coli* MG1655 (which does not contain *bsh* genes within its genome), along with two forms of *E. coli* MG1655 into which *bsh* genes had been cloned using pBKminiTn7GM2 under the control of the P44 promotor[25] (see online supplementary methods 1.7). *C. difficile* spores from three different ribotypes (a non-toxigenic ribotype, 010 (strain DS1684), and two toxigenic ribotypes,

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

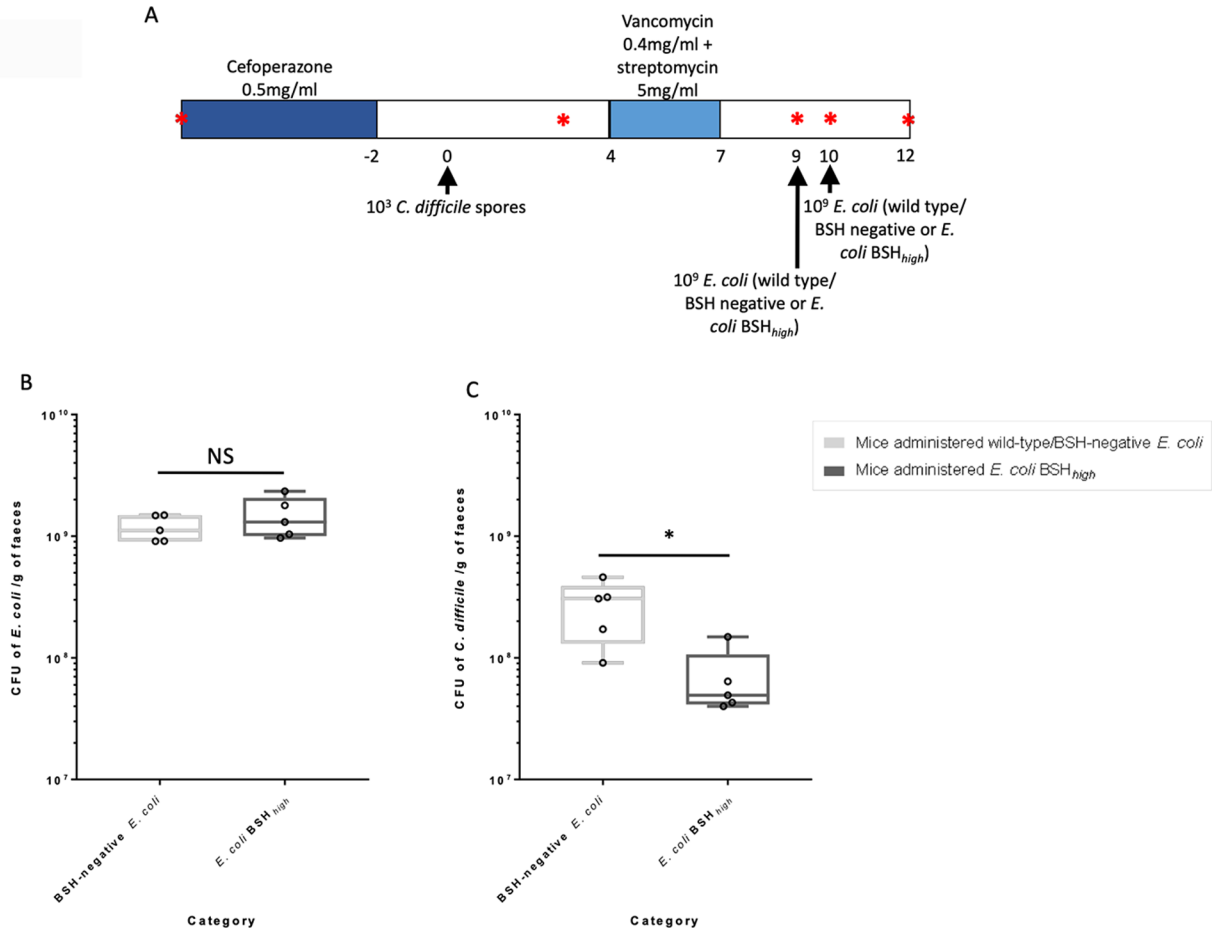


Figure 1 Impact of BSH on a mouse model of rCDI. A mouse model of rCDI/FMT was used that was adapted from a previously described model.[26] (A) Protocol for mouse model (*: stool samples taken for *C. difficile* and/or *E. coli* counts); (B) Counts of administered *E. coli* on day 12; (C) Total vegetative counts of *C. difficile* on day 12. CFU counts as established from plate counts of serial dilutions of faecal supernatant (*, p<0.05, Mann-Whitney U test). BSH, bile salt hydrolases; CFU, colony-forming unit; FMT, faecal microbiota transplant; rCDI, recurrent *C. difficile* infection.

012 (strain CD630) and 027 (strain R20291)) were resuspended in supernatant in triplicate, and an OD_{600} reading taken on a microplate reader at time zero (adjusted to OD_{600} of ~0.1 with supernatant/sBHI mix), and again after overnight incubation. An increased OD_{600} reading after overnight incubation was interpreted as indicating that spores had undergone germination and had grown as vegetative cells.[11] Additionally, UPLC-MS was performed on batch culture supernatants to establish bile acid profiles (see online supplementary methods 1.5.2), and BSH activity assays were performed on spent supernatant from selected batch cultures.

Recurrent *C. difficile* mouse model

Wild-type C57BL/6 mice (8–10 weeks old; female) were purchased from Envigo (Huntingdon, UK) and acclimatised for 1 week before use. Mice were housed five per cage in individually ventilated cages with autoclaved food (RM1, Special Diet Services, Essex, UK), bedding (Aspen chip two bedding, Northeastern Products Corporation (NEPCO), Warrensburg, New York) and water (provided ad libitum). Mice were subjected to a 12 hours light and 12 hours dark cycle at 20°C–22°C.

We adapted a previously published mouse model of rCDI/FMT[26] (figure 1A). Mice were initially given cefoperazone 0.5 mg/mL (Melford, Ipswich, UK) in their drinking water for 5 days (day −7 to day −2), followed by challenge with 10^3 *C. difficile* spores by oral gavage on day 0. Mice were then treated with vancomycin 0.4 mg/mL together with streptomycin 5 mg/mL (both Melford, Ipswich, UK) in their drinking water for 3 days (days 4–7), followed by autoclaved antibiotic-free water for the remainder of the experiment. On both days 9 and 10, mice were fed by oral gavage either with ~10^9 colony-forming units (CFUs) of wild-type *E. coli* (n=5) or *E. coli* BSH_{high} (n=5) (see online supplementary methods 1.7). Serial faecal samples were collected and *C. difficile* total viable counts (TVCs) quantified until *E. coli* colonisation began to decline (see online supplementary methods 1.9). Administered *E. coli* were quantified by plating on MacConkey agar plates supplemented with rifampicin 50 µg/mL (Melford, Ipswich, UK). Mice were not fasted before oral gavages and all interventions were performed during the light cycle.

Statistical analysis

This is summarised in online supplementary methods 1.10.

RESULTS

Successful FMT for rCDI is associated with restoration of BSH-producing gut microbial community members from all BSH groups

We used microbial sequencing to evaluate whether FMT for rCDI was associated within an increase in BSH-producing microbes within the faecal microbiota. Further analysis of metataxonomic

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

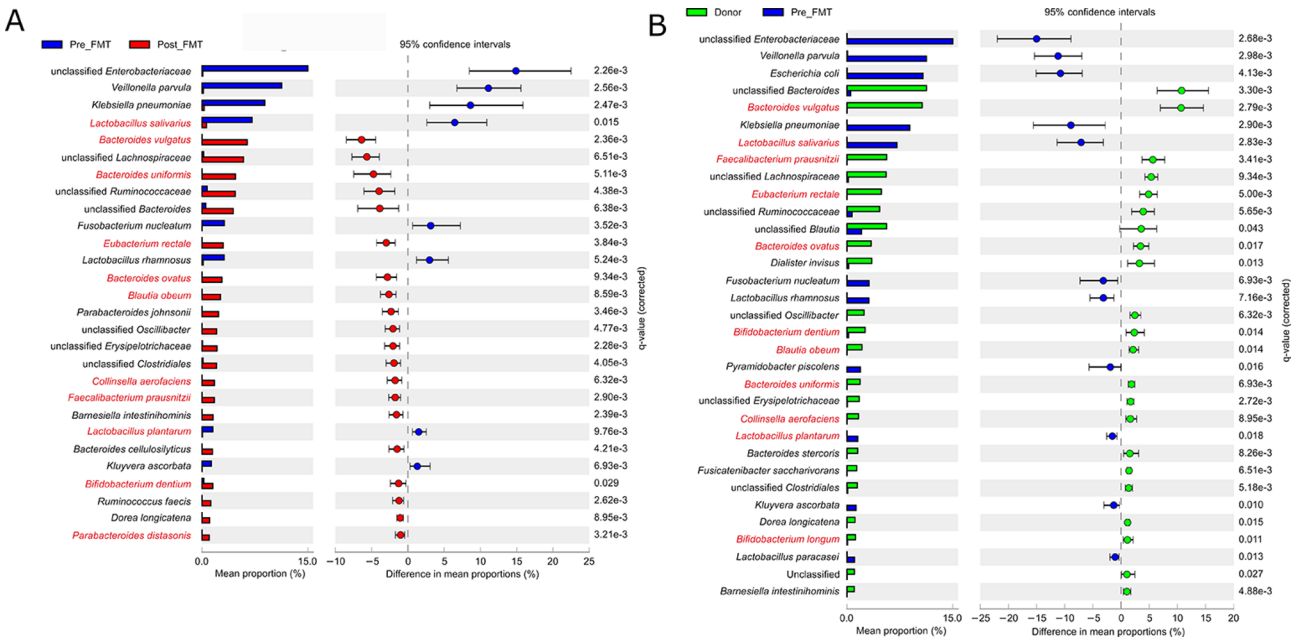


Figure 2 Species differences in 16S rRNA gene sequencing data in patients with rCDI compared with donor or post-FMT. Extended error bar plots, with bacterial species changing significantly measured by White's non-parametric test with Benjamini-Hochberg correction, using threshold of differences between mean proportions >1%. (A) Donor versus pre-FMT; (B) Pre-FMT versus post-FMT. Names in red: known BSH-producing bacteria. BSH, bile salt hydrolases; FMT, faecal microbiota transplant; rCDI, recurrent *C. difficile* infection.

data is provided in online supplementary results, figures 3–5 and table 2.

Bacterial species enriched in the faecal microbiota of healthy donors compared with pre-FMT samples were characterised by a range of BSH-producing organisms, including members of group 1 (*Bacteroides ovatus*, $q=0.017$; *Bacteroides uniformis*, $q=0.007$), group 2 (*Bifidobacterium dentium*, $q=0.014$; *Collinsella aerofaciens*, $q=0.009$; *Bifidobacterium longum*, $q=0.011$) and group 3 (*Bacteroides vulgatus*, $q=0.003$; *Faecalibacterium prausnitzii*, $q=0.003$; *Eubacterium rectale*, $q=0.005$; *Blautia obeum*, $q=0.014$) (figure 2A). Similarly, bacterial species enriched in the post-FMT faecal microbiota compared with those pre-FMT also included members of all BSH groups, including group 1 (*Bacteroides uniformis*, $q=0.005$; *Bacteroides ovatus*, $q=0.009$; *Parabacteroides distasonis*, $q=0.003$), group 2 (*Collinsella aerofaciens*, $q=0.006$; *Bifidobacterium dentium*, $q=0.029$) and group 3 (*Bacteroides vulgatus*, $q=0.002$; *Eubacterium rectale*, $q=0.004$; *Blautia obeum*, $q=0.009$; *Faecalibacterium prausnitzii*, $q=0.003$) (figure 2B).

Using Piphillin to infer metagenomic content from metataxonomic data, there were a significantly reduced proportion of sequences predicted to represent both BSH (see online supplementary figure 6A) and 7-α-dehydroxylase (see online supplementary figure 6B) in pre-FMT samples compared with donors, but a significant increase in both in post-FMT samples compared with pre-FMT ($q<0.01$, White's non-parametric test with Benjamini-Hochberg false discovery rate (FDR)).

Successful FMT for rCDI is associated with restoration of normal gut bile acid profiling, including a sustained reduction in TCA

We performed faecal bile acid profiling to assess the effect of FMT for rCDI on key bile acids known to affect the ability of *C. difficile* to undergo germination or vegetative growth.

On multivariate analysis of UPLC-MS bile acid profiling data, unsupervised principal component analysis demonstrated clustering of donor and post-FMT samples, but clear separation of both groups from pre-FMT samples (figure 3A). Supervised analysis was performed with orthogonal projections to latent structures discriminant analysis (OPLS-DA) to analyse the features responsible for discrimination between donor and pre-FMT groups (see online supplementary figure 7A), and between pre-FMT and post-FMT groups (figure 3B). Discriminatory feature identification was performed from OPLS-DA model data via S-plot, with pre-FMT samples showing an enrichment in primary bile acids (including both conjugated and unconjugated forms) and loss of secondary bile acids as compared with post-FMT and healthy donor samples (figure 3C, see online supplementary figure 7B). OPLS-DA model validation was performed using CV analysis of variance (see online supplementary table 3).

Univariate analysis supported these findings (see online supplementary figure 8). Pre-FMT samples demonstrated enrichment in TCA and loss of DCA compared with healthy donor samples ($p<0.01$, Mann-Whitney U test), while post-FMT samples were characterised by restoration of both bile acids back to levels comparable to donors ($p<0.001$, Wilcoxon signed rank-sum test).

Integration of metataxonomic and bile acid profiling data

rCCA modelling was used to integrate metataxonomic and bile acid profiling data. The unit representation plot demonstrated marked separation of pre-FMT and post-FMT samples, but considerable overlap between donor and post-FMT samples (figure 4A). A correlation circle plot demonstrated negative correlations between levels of TCA and the abundance of

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

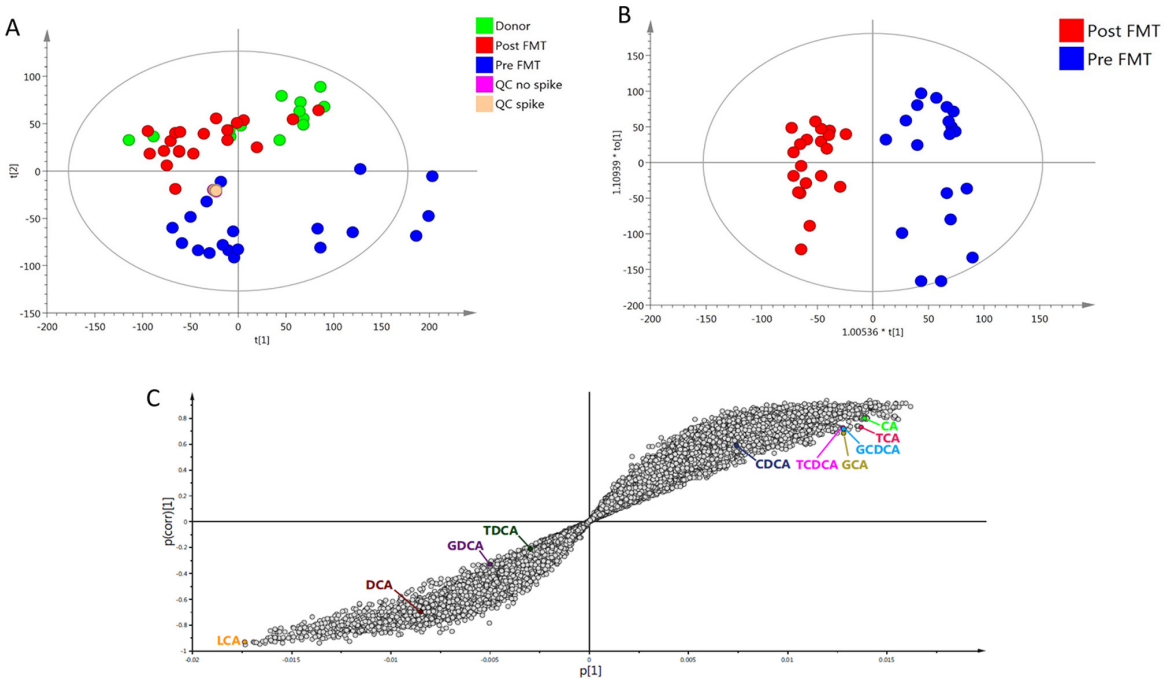


Figure 3 Effect of FMT for rCDI on stool bile acid profiles. Assessed via multivariate analysis of UPLC-MS bile acid profiling data. (A) PCA scores plot; (B) OPLS-DA scores plot, comparing pre-FMT and post-FMT samples; (C) OPLS-DA S-plot of pre-FMT versus post-FMT data. FMT, faecal microbiota transplant; OPLS-DA, orthogonal projections to latent structures discriminant analysis; PCA, principal component analysis; QC, quality controls; rCDI, recurrent *C. difficile* infection; UPLS-MS, ultra performance liquid chromatography mass spectrometry.

the bacterial genera *Bacteroides* and *Blautia*, both known to include representative BSH-producing organisms that were significantly increased after FMT in our metaxonomic data (figure 4B). In addition, there was positive correlation between the genus *Clostridium* cluster XIVa (known to contain 7-α-dehydroxylase producing organisms[27 28]) and the secondary bile acids DCA and lithocholic acid (figure 4B).

Gut *bsh* gene copy number and BSH enzyme activity is restored by successful FMT for rCDI

Having established an association between restoration of BSH-producing organisms into the gut microbiota post-FMT and recovery of premorbid stool bile acid profiles, we investigated the effect of FMT for rCDI on BSH expression and activity.

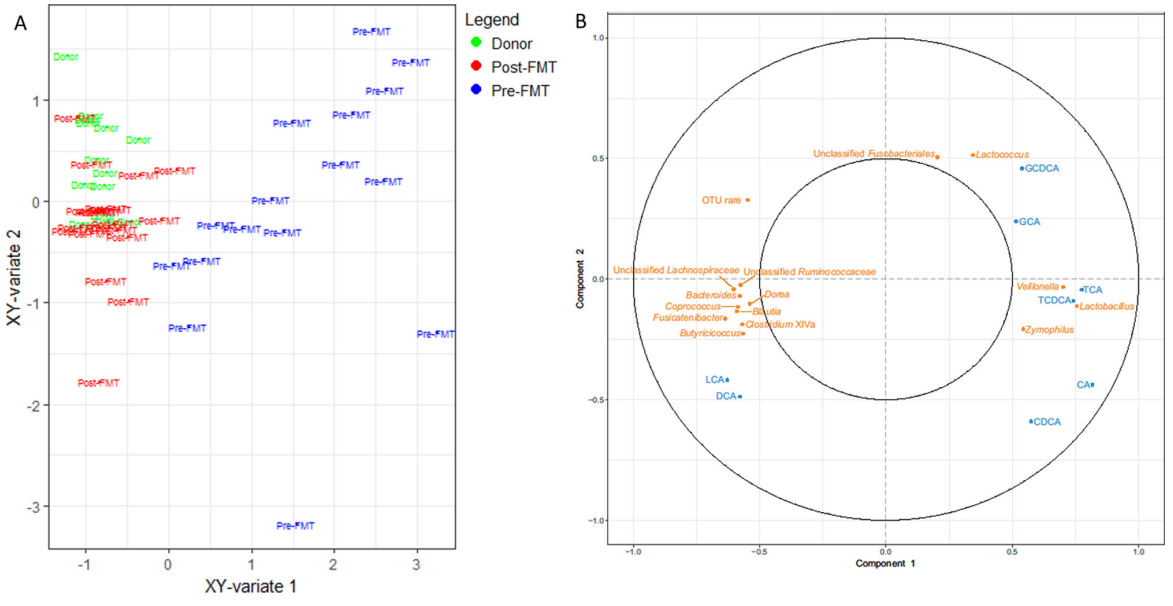


Figure 4 Regularised CCA (rCCA) model correlating 16S rRNA gene sequencing data (genus level) and bile acid data. (A) Unit representation plot for the two canonical variables (metataxonomics and stool bile acids); (B) Correlation circle plot between pre-FMT and post-FMT samples. Bile acids are shown in blue and bacterial genera are shown in orange. FMT, faecal microbiota transplant.

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

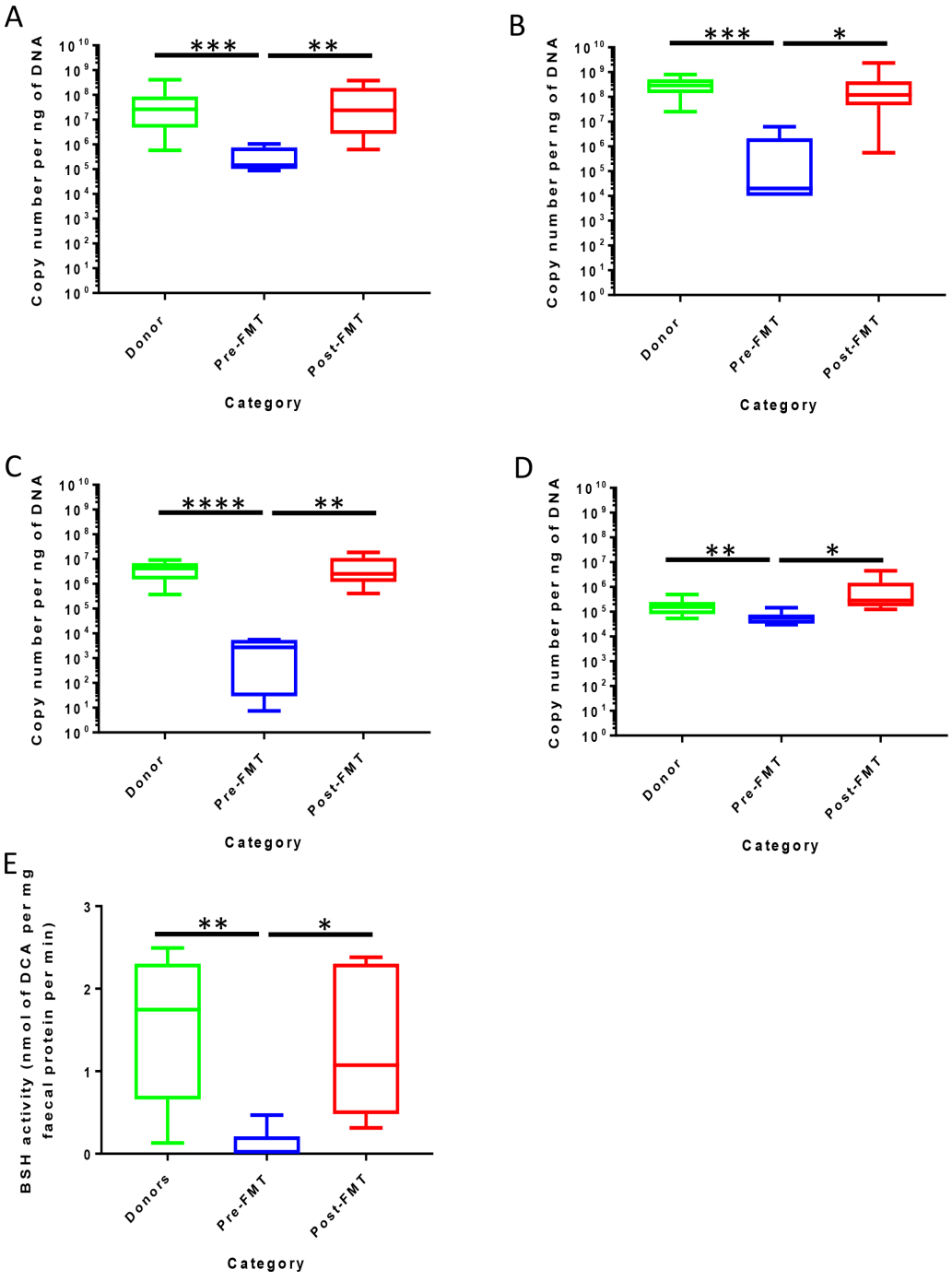


Figure 5 Effect of FMT on bile-metabolising enzyme gene copy number and BSH enzyme activity. (A) *bsh* group 1A; (B) *bsh* group 1B; (C) *bsh* group 3C; (D) *bai*CD operon of 7-α-dehydroxylase; (E) BSH enzyme activity within faecal supernatant (*, p<0.05; **, p<0.01; ***; p<0.001; ****, p<0.0001; Mann-Whitney U test for donors versus pre-FMT, Wilcoxon signed rank-sum test for pre-FMT vs post-FMT). BSH, bile salt hydrolases; FMT, faecal microbiota transplant.

bsh gene copy number was significantly reduced in pre-FMT samples compared with healthy donors across a range of *bsh* gene groups (p<0.001, Mann-Whitney U test, figure 5A–C). Successful FMT was associated with significant enrichment in copy number of all *bsh* gene groups assayed (p<0.05, Wilcoxon signed rank-sum test, figure 5A–C) to levels similar to that of healthy donors. Similarly, copy numbers of the *bai*CD operon (encoding an enzyme that contributes to 7-α-dehydroxylation[29]) were significantly lower in pre-FMT samples compared with those of healthy donors (p<0.01), but were also significantly enriched post-FMT (p<0.05, figure 5D). Gene copy number for *bai*CD in donors and post-FMT was noted to be markedly lower than for each *bsh* gene assayed for the same participants. Stool BSH enzyme activity showed the same pattern as for *bsh* gene copy number (figure 5E).

To further explore the timescale of gut bile acid/BSH changes post-FMT, we analysed serial stool samples collected from patients in a randomised trial of colonoscopy versus capsule FMT as treatment for rCDI,[15] together with donor samples and FMT slurry itself. This demonstrated that BSH activity was restored (and stool TCA levels greatly reduced) to levels comparable to donors within a week of successful FMT, and was

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

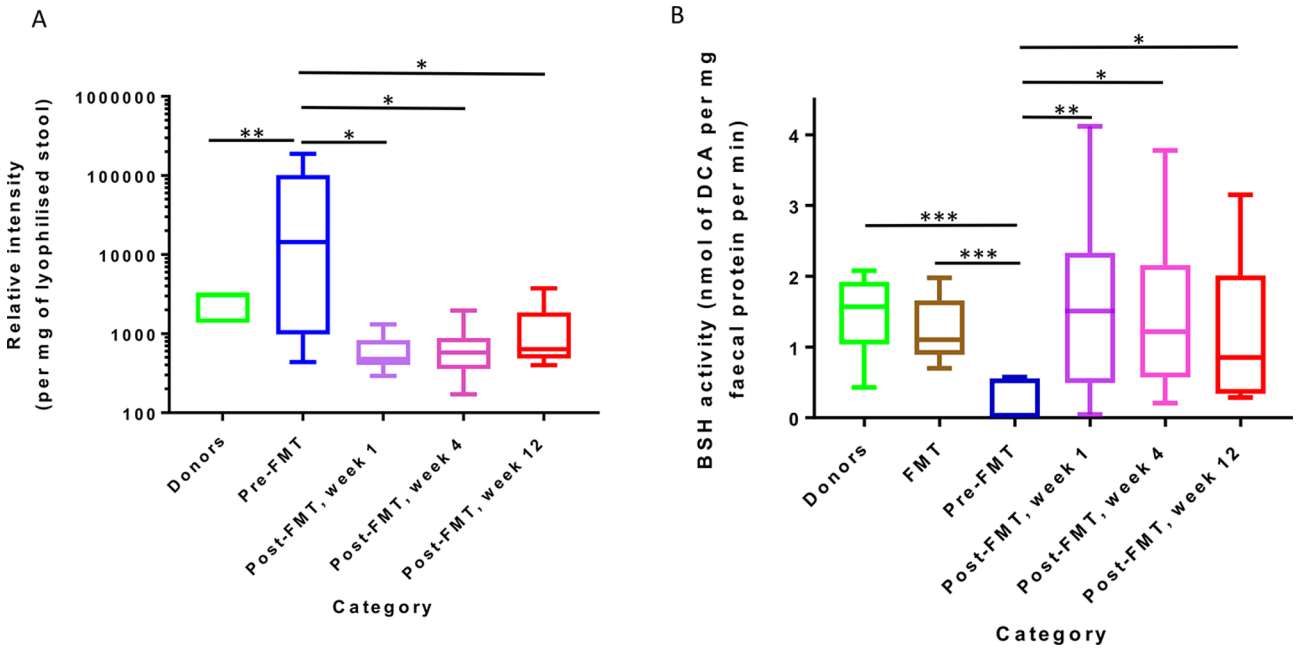


Figure 6 Dynamics of changes in taurocholic acid and BSH activity after FMT for rCDI. Assessed via analysis of taurocholate levels in stool ((A) as assessed by UPLC-MS) and BSH activity in stool and FMT slurry ((B) assessed via precipitation assay). Samples were collected from patients (and their matched donors, along with FMT slurry itself) in a randomised trial of colonoscopy versus capsule FMT as treatment for rCDI[15] (*, p<0.05; **, p<0.01; ***, p<0.001; Mann-Whitney U test for donors/FMT slurry versus pre-FMT, Friedman test with Benjamini-Hochberg FDR for pre-FMT vs post-FMT). BSH, bile salt hydrolases; DCA, deoxycholic acid; FMT, faecal microbiota transplant; rCDI, recurrent *C. difficile* infection; UPLC-MS, ultra performance liquid chromatography mass spectrometry.

maintained at these levels at 4 weeks and 12 weeks post-FMT (p<0.05, Friedman test with Benjamini-Hochberg FDR; figure 6A,B). Similarly, BSH activity was significantly reduced in pre-FMT samples compared with either donor stool or FMT slurry (p<0.01, Mann-Whitney U test; figure 6B).

BSH activity alone is sufficient to fully suppress TCA-mediated *C. difficile* germination in vitro

Our data demonstrated an association between successful FMT, breakdown of TCA, and restoration of gut BSH-producing microorganisms and BSH functionality. To further explore whether these changes were purely associative–or represented a true mechanistic pathway–we performed *C. difficile* batch culture germination experiments.

We prepared spent culture supernatants by incubating bacteria of interest in broth with 1% w/v TCA. After overnight incubation, cultures were centrifuged and filter-sterilised. *C. difficile* spores were incubated in sBHI broth supplemented with the spent culture supernatant. Using this set-up, *C. difficile* spores incubated with spent culture supernatants without BSH activity would have TCA available to stimulate germination and therefore grow, while *C. difficile* spores incubated with spent culture supernatants with BSH activity would not have TCA available to stimulate germination and would not grow.

Initially, *C. difficile* spores were incubated with spent supernatant from BSH-expressing microorganisms that had been incubated with TCA. The microorganisms selected were those which had been shown to be reduced in mean proportion in the gut microbiota of pre-FMT patients in comparison to donors and/or post-FMT samples, and which collectively represented most BSH groups. For all *C. difficile* ribotypes assayed, supernatant from the broth of each of the BSH-producing microbes assayed significantly reduced *C. difficile* germination (p<0.0001) (figure 7A). As a control, we used spent supernatant from TCA-supplemented broth in which vegetative *C. difficile* had been cultured (strain DS1864); this failed to affect *C. difficile* germination.

We assessed whether BSH alone could mediate this inhibition, or if it reflected an alternative aspect of bacterial metabolism within the cultures. For subsequent batch cultures, strains of interest included wild-type *E. coli* (which lacks a *bsh* gene), or two forms of *E. coli* into which *bsh* genes had been cloned (ie, '*E. coli* BSH_{low}', containing a *bsh* gene with narrow substrate range against conjugated bile acids; and '*E. coli* BSH_{high}', containing a *bsh* gene with high glycine and taurine-deconjugating activity) (see online supplementary methods 1.7). While spent supernatant from the culture of wild-type/BSH negative *E. coli* did not affect the ability of *C. difficile* to undergo germination, that from both forms of *bsh* gene-expressing *E. coli* significantly reduced *C. difficile* germination across all ribotypes tested (p<0.0001, Student's t-test) (figure 7B). *C. difficile* germination was significantly lower for all three ribotypes when incubated in TCA-supplemented supernatant from an *E. coli* BSH_{high} batch culture as compared with *E. coli* BSH_{low} (p<0.05).

C. difficile spores were also cultured in spent supernatant from *C. scindens* which had been incubated with TCA, either by itself or also in co-culture with BSH-expressing *E. coli*. *C. scindens* spent supernatant did not affect *C. difficile* germination ability by itself, but germination was significantly reduced when *C. scindens* was co-incubated with BSH-expressing *E. coli* (p<0.01) (figure 7C).

Analysis by UPLC-MS confirmed that TCA-supplemented batch culture media–which included supernatant from wild-type/BSH-negative *E. coli*, vegetative *C. difficile* and *C. scindens* culture–had high TCA:CA ratios, consistent with little/absent BSH activity within these batch cultures

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

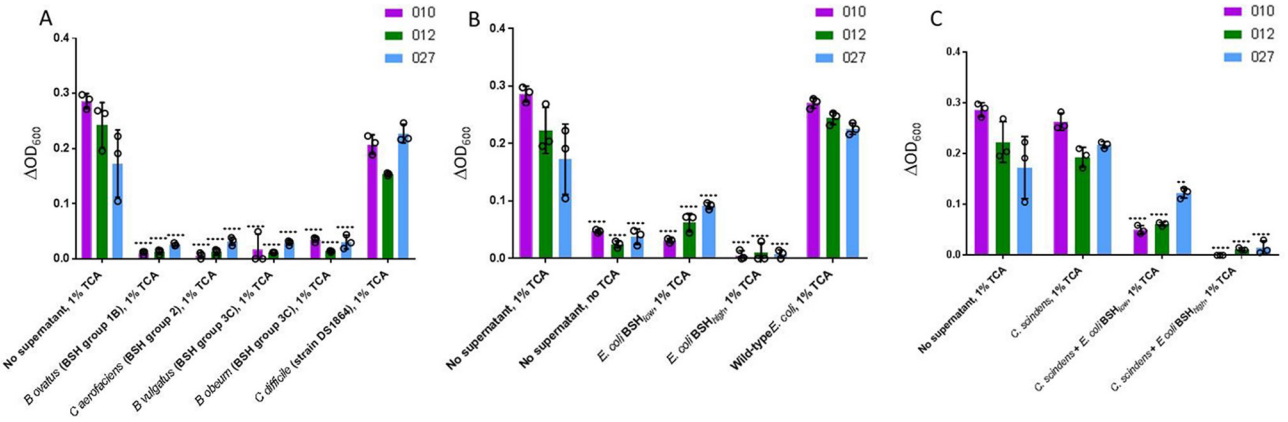


Figure 7 *C. difficile* batch cultures. Changes in spectrophotometer reading (ΔOD_{600}) after overnight incubation of *C. difficile* spores (three ribotypes assayed: 010, 012, 027) in sBHI ±TCA in which bacterial species of interest had been cultured for 24 hours. *C. difficile* spores in sBHI supplemented with 1% TCA ('No supernatant, 1% TCA') was used as positive control in all cases; statistical testing shown was performed relative to this sample for the particular ribotype under assessment. (A) Batch cultures of BSH-producing microbial species found to be affected by FMT in metataxonomic analysis, and vegetative *C. difficile*. (B) Batch cultures of native *E. coli,* and two forms of *E. coli* into which *bsh* genes had been cloned *(E. coli* BSH_{low}=*E. coli* expressing BSH with low deconjugation ability; *E. coli* BSH_{high}=*E. coli* expressing BSH with high deconjugation activity). (C) Batch cultures of *C. scindens*±BSH expressing *E. coli* (**, p<0.01; ****, p<0.0001; analysis of variance with multiple group comparisons, Benjamini-Hochberg correction). Key: 010 is a non-toxigenic *C. difficile* ribotype, while 012 and 027 are both toxigenic ribotypes. BHI, brain heart infusion; BSH, bile salt hydrolases; TCA, taurocholic acid.

(see online supplementary figure 9A). Low TCA:CA ratios were noted for all other batch cultures, indicating the presence of BSH activity. All batch culture experiments in which *C. difficile* germination was suppressed were characterised by undetectable levels of DCA within supernatant (other than batch cultures containing *C. scindens* and *bsh*-expressing *E. coli*, where DCA was detectable). Sterile-filtered spent supernatant from batch cultures of BSH-producing microorganisms from groups 1, 2 and 3 was found to have BSH activity comparable to that found in healthy human stool (see online supplementary figure 9B).

BSH limits further recurrence of CDI in a mouse model of rCDI

To investigate the hypothesis that BSH-producing bacteria limit further recurrence of disease in subjects with rCDI, we administered ~10^9 CFU of either wild-type/BSH-negative *E. coli* or *E. coli* BSH_{high} (n=5 in both groups) into a mouse model of rCDI shortly after completion of vancomycin (figure 1A). We used this particular protocol to aim to recapitulate the dynamics of FMT administration to patients with rCDI. Colonisation of all mice with *C. difficile* was demonstrated at 3 days post-*C. difficile* spore administration (see online supplementary figure 10A), and plate counts were used to confirm the titre of *E. coli* administered to mice (see online supplementary figure 10B). *E. coli* colonisation of the gut of mice was assessed by plate counts on selective media; we identified that on day 12 (ie, 3 days after initial *E. coli* administration), *E. coli* colonisation was at comparable, high levels in both groups of mice (mean CFU per gram of faeces of 1.49×10^9 in mice administered *E. coli* BSH_{high} vs 1.18×10^9 in mice administered wild-type *E. coli*, p>0.05, Mann-Whitney U test) (figure 1B).

On day 12, *C. difficile* TVCs were significantly reduced in the mice administered *E. coli* BSH_{high} in comparison to those administered wild-type/BSH-negative *E. coli* (figure 1C; mean CFU per gram of faeces of 6.92×10^7 vs 2.70×10^8, respectively; p<0.05, Mann-Whitney U test), equating to a ~70% reduction in *C. difficile* total vegetative cell counts.

DISCUSSION

In this study, we demonstrate for the first time that a key mechanism underlying the efficacy of FMT in treating rCDI is restoration of gut microbiota BSH functionality. Analysis of human samples illustrate that this function is restored early after FMT and maintained throughout follow-up. In addition, data from batch cultures and mouse modelling show that the BSH-mediated hydrolysis of the major *C. difficile* germinant, TCA, is sufficient to fully suppress *C. difficile* germination, and limits further recurrence of disease within the setting of rCDI. Targeted restoration of gut BSH function is a novel therapeutic approach for rCDI that avoids the risks associated with FMT.

The potential role of gut microbiota-host bile acid interactions in CDI pathogenesis has been an area of interest since initial studies in vitro established that various bile acids differentially affected the ability of *C. difficile* to undergo germination and vegetative growth.[11 12 30–33] Furthermore, we have also recently demonstrated that successful FMT for rCDI is associated with stimulation of farnesoid X receptor (FXR) signalling,[34] which in itself appears to impact the bile acid milieu and consequently gut microbiota of the small intestine.[35] Subsequent studies have demonstrated that the gut of germ-free[36] and antibiotic-treated mice[36–38] –as well as chemostat models of CDI[8] or humans with rCDI[39 40]–have enrichment of stool primary bile acids (particularly conjugated versions) and loss of secondary bile acids, with bile acid homeostasis being restored in patients with rCDI through FMT.[39] Exposure of *C. difficile* spores to the bile acid milieu found in antibiotic-treated mouse caecum[37 38] or human stool pre-FMT[41] was sufficient to cause spore germination, while that of the non-antibiotic-treated mouse caecum[37 38] or human stool post-FMT[41] prevented germination and vegetative growth of *C. difficile*.

Rodent studies have demonstrated that 7-α-dehydroxylase-producing organisms (in particular, *C. scindens*) partly protected the host against the development of CDI.[42 43] However, to date, there is not sufficient evidence to demonstrate that 7-α-dehydroxylase is responsible for the efficacy of FMT for rCDI. For example,

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

a mouse model of CDI was successfully treated with a mixture of six bacteria, none of which are recognised to contain 7-α-dehydroxylase activity.[44] Furthermore, *bai*CD gene abundance is not different in the stool of patients with CDI and *C. difficile*-negative patients (after correction for total bacterial load), and the *bai*CD gene is not consistently detectable in stool after successful FMT for rCDI;[45] a study using microbial sequencing and culture demonstrated comparable results.[46] As such, our key area of focus in this study was regarding the dynamics of BSHs in rCDI and the impact of FMT on BSH functionality. Allegretti and coauthors previously demonstrated that predicted BSH functionality was significantly reduced in the stool microbiota of patients with rCDI compared with healthy controls or those with first CDI.[14] Our data demonstrate the restoration of BSH-producing microorganisms and associated BSH functionality post successful FMT for rCDI from the very low levels found pre-FMT back to levels similar to that of donors. This result is coupled with the loss of primary conjugated bile acids, and particularly that of the progerminant TCA.

In patients with and animal models of rCDI, few vegetative cells of *C. difficile* remain in the gut after completion of vancomycin.[26] For further recurrence to occur, TCA-mediated germination of *C. difficile* is likely to be a key contributory factor. Our batch culture and mouse model data demonstrate that hydrolysis of TCA by BSH is sufficient to significantly limit *C. difficile* counts in rCDI. To date, no studies have been reported in mice that demonstrated restoration of 7-α-dehydroxylase activity alone could reduce *C. difficile* growth. While we have shown that degradation of TCA is the major mechanism by which restoration of BSH limits further recurrence in rCDI, an additional mechanism may be that BSH creates a larger pool of deconjugated primary bile acids, the substrate for further gut bacterial enzyme degradation and conversion of primary into secondary bile acids within the colon (see online supplementary discussion).

Given the drawbacks that currently exist regarding FMT clinically and the acknowledged need for more refined, targeted therapies, we propose that the administration of BSH-producing gut microbiota members—or the administration of purified BSH enzyme— merits further evaluation as an alternative CDI treatment strategy. Administration of a microbial community containing BSH-producing bacteria (such as those identified in this study) might be expected—like FMT—to require a single administration only, given the apparent ability of these organisms to easily colonise the gut; in contrast, to attain comparable efficacy, delivery of purified BSH enzyme is likely to require a more prolonged administration. Furthermore, while we have demonstrated proof of concept of 'BSH therapy' in a batch culture and mouse model setting, this would evidently merit evaluation within a clinical trial setting before it could be considered further as treatment of human patients with rCDI. It is also important to acknowledge that FMT may provide additional mechanisms of efficacy in treating rCDI (eg, potentially by competitive niche exclusion, related to the near-complete restoration of a diverse gut microbial community with high resilience[47 48]), and such benefits may be missed through the use of BSH supplementation alone.

As well as a novel treatment for CDI, BSH supplementation may be a potential novel strategy for prevention of CDI in those at high risk, for example, patients likely to require prolonged antibiotic courses. In addition, FMT has a small but appreciable failure rate, and there is currently no rational targeted biological means by which donors are selected. Assays of stool from potential donors for BSH-producing organisms and/or BSH functionality may be one such means to achieving this aim.

In conclusion, we provide evidence for the first time that microbial BSHs are a key mediator of the efficacy of FMT for rCDI. This adds to other recent data demonstrating that FMT functions by reversing a 'metabolic dysbiosis', including through the restoration of the short chain fatty acid valerate, which potently inhibits the growth of *C. difficile* without any apparent adverse effect on gut commensal bacteria.[8] Furthermore, these data add further to the growing body of evidence demonstrating the central significance of gut microbiota-bile acid interactions in colonisation resistance, and demonstrate that targeted restitution of BSH may be a novel therapy for or preventative strategy against CDI that avoids the risks of antimicrobial resistance.

Author affiliations
[1]Division of Integrative Systems Medicine and Digestive Disease, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK
[2]Division of Gastroenterology, Hepatology and Endoscopy, Brigham and Women's Hospital, Boston, Massachusetts, USA
[3]Harvard Medical School, Harvard University, Boston, Massachusetts, USA
[4]Division of Gastroenterology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada
[5]Division of Infectious Diseases/ GI Diseases Research Unit Wing, Department of Medicine, Kingston General Hospital, Queen's University, Kingston, Ontario, Canada
[6]APC Microbiome Institute, University College Cork, Cork, Ireland
[7]School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland
[8]School of Pharmacy, University College Cork, Cork, Ireland
[9]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, UK
[10]School of Biosciences, Cardiff University, Cardiff, UK

Acknowledgements *bsh* gene-expressing *E. coli* were provided by SAJ and CGMG, APC Microbiome Institute, University College Cork, Cork, Ireland. The authors wish to thank Douglas N Rees for laboratory technical support, Dr Stephen R Atkinson for support with data analysis, Mr Chris Mack for support with the mouse work and Mr Matt Emberg for help with administration related to sample preparation and shipment.

Contributors BHM and JAKM were involved in all aspects of experimental work and data analysis; JRA, DK and EOP provided samples and contributed to data analysis; AP and EH provided analysis of mass spectrometry data; GFB and DK performed qPCR and data analysis; SAJ and CGMG provided bacteria and aided analysis of batch culture experiments; BHM, JAKM, IG-M and TBC performed the mouse experiments; HRTW, MRT and JRM contributed to study design and provided overall supervision of experimental work and data analysis. All authors contributed to and approved the final manuscript.

Funding The Division of Integrative Systems Medicine and Digestive Disease at Imperial College London receives financial support from the National Institute of Health Research (NIHR) Imperial Biomedical Research Centre (BRC) based at Imperial College Healthcare NHS Trust and Imperial College London. This paper is independently funded by the NIHR BRC, and the views expressed in this publication are those of the authors, and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health. BHM is the recipient of a Medical Research Council (MRC) Clinical Research Training Fellowship (grant reference: MR/R000875/1). DK receives research funding from Alberta Health Services and University of Alberta Hospital Foundation. EOP is supported by the Southern Ontario Academic Medical Organization, and National Institutes of Health Grant (R33/AI21575). SAJ and CGC are funded by SFI 12/RC/2273 to APC Microbiome Institute and SFI 16/ERA-HDHL/3358 (to SAJ). TBC is a Sir Henry Dale Fellow jointly funded by the Wellcome Trust and Royal Society (grant 107660/Z/15Z).

Competing interests JRA consults for Finch Therapeutics. DK has received research funding from Rebiotix. EOP is a co-founder of Nubiyota and serves on its scientific advisory board. All other authors declared no conflict of interests.

Patient consent for publication Not required.

Ethics approval This study received approval from the UK National Research Ethics Centres (13/LO/1867) and REB approval from all participating institutions. Mouse experiments were performed under the authority of the UK Home Office outlined in the Animals (Scientific Procedures) Act 1986 after ethical review by the Imperial College London Animal Welfare and Ethical Review Body (PPL 70/7969). Standards articulated in the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were adhered to.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution 4.0 Unported (CC BY 4.0) license, which permits

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.

others to copy, redistribute, remix, transform and build upon this work for any purpose, provided the original work is properly cited, a link to the licence is given, and indication of whether changes were made. See: https://creativecommons.org/licenses/by/4.0/.

REFERENCES

1 Lawson PA, Citron DM, Tyrrell KL, *et al*. Reclassification of Clostridium difficile as Clostridioides difficile (Hall and O'Toole 1935) Prévot 1938. *Anaerobe* 2016;40:95–9.
2 Ma GK, Brensinger CM, Wu Q, *et al*. Increasing Incidence of Multiply Recurrent Clostridium difficile Infection in the United States: A Cohort Study. *Ann Intern Med* 2017;167:152–8.
3 King A, Mullish BH, Williams HRT, *et al*. Comparative epidemiology of Clostridium difficile infection: England and the USA. *Int J Qual Health Care* 2017;29:785–91.
4 Mullish BH, Quraishi MN, Segal JP, *et al*. The use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications: joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut* 2018;67:1920–41.
5 Mattila E, Uusitalo-Seppälä R, Wuorela M, *et al*. Fecal transplantation, through colonoscopy, is effective therapy for recurrent *Clostridium difficile* infection. *Gastroenterology* 2012;142:490–6.
6 Mullish BH, Williams HR. Obstacles to establishing an NHS faecal transplant programme. *BMJ* 2015;351:h6043.
7 Ott SJ, Waetzig GH, Rehman A, *et al*. Efficacy of sterile fecal filtrate transfer for treating patients with clostridium difficile infection. *Gastroenterology* 2017;152:799–811.
8 McDonald JAK, Mullish BH, Pechlivanis A, *et al*. Inhibiting growth of *Clostridioides difficile* by restoring valerate, produced by the intestinal microbiota. *Gastroenterology* 2018;155:1495–507.
9 Zuo T, Wong SH, Lam K, *et al*. Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome. *Gut* 2018;67:634–43.
10 Draper LA, Ryan FJ, Smith MK, *et al*. Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation. *Microbiome* 2018;6:220.
11 Sorg JA, Sonenshein AL. Bile salts and glycine as cogerminants for Clostridium difficile spores. *J Bacteriol* 2008;190:2505–12.
12 Thanissery R, Winston JA, Theriot CM. Inhibition of spore germination, growth, and toxin activity of clinically relevant C. difficile strains by gut microbiota derived secondary bile acids. *Anaerobe* 2017;45:86–100.
13 Jones BV, Begley M, Hill C, *et al*. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci U S A* 2008;105:13580–5.
14 Allegretti JR, Kearney S, Li N, *et al*. Recurrent Clostridium difficile infection associates with distinct bile acid and microbiome profiles. *Aliment Pharmacol Ther* 2016;43:1142–53.
15 Kao D, Roach B, Silva M, *et al*. Effect of Oral Capsule– vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent *Clostridium difficile* Infection. *JAMA* 1985;2017:318.
16 Kelly CR, Kahn S, Kashyap P, *et al*. Update on Fecal Microbiota Transplantation 2015: Indications, Methodologies, Mechanisms, and Outlook. *Gastroenterology* 2015;149:223–37.
17 Mullish BH, Pechlivanis A, Barker GF, *et al*. Functional microbiomics: evaluation of gut microbiota-bile acid metabolism interactions in health and disease. *Methods* 2018;149:49–58.
18 Illumina. 16S Metagenomic Sequencing Library Preparation. https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html (Accessed 27 Nov 2017).
19 Iwai S, Weinmaier T, Schmidt BL, *et al*. Piphillin: improved prediction of metagenomic content by direct inference from human microbiomes. *PLoS One* 2016;11:e0166104.
20 Sarafian MH, Lewis MR, Pechlivanis A, *et al*. Bile acid profiling and quantification in biofluids using ultra-performance liquid chromatography tandem mass spectrometry. *Anal Chem* 2015;87:9662–70.
21 Wells JE, Williams KB, Whitehead TR, *et al*. Development and application of a polymerase chain reaction assay for the detection and enumeration of bile acid 7alpha-dehydroxylating bacteria in human feces. *Clin Chim Acta* 2003;331:127–34.
22 Ling WH, Korpela R, Mykkänen H, *et al*. Lactobacillus strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. *J Nutr* 1994;124:18–23.
23 Tanaka H, Hashiba H, Kok J, *et al*. Bile salt hydrolase of Bifidobacterium longum-biochemical and genetic characterization. *Appl Environ Microbiol* 2000;66:2502–12.
24 Smith K, Zeng X, Lin J, *et al*. Discovery of bile salt hydrolase inhibitors using an efficient high-throughput screening system. *PLoS One* 2014;9:e85344.
25 Joyce SA, MacSharry J, Casey PG, *et al*. Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. *Proc Natl Acad Sci U S A* 2014;111:7421–6.
26 Seekatz AM, Theriot CM, Molloy CT, *et al*. Fecal microbiota transplantation eliminates clostridium difficile in a Murine model of relapsing disease. *Infect Immun* 2015;83:3838–46.
27 Kitahara M, Takamine F, Imamura T, *et al*. Assignment of Eubacterium sp. VPI 12708 and related strains with high bile acid 7alpha-dehydroxylating activity to Clostridium scindens and proposal of Clostridium hylemonae sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* 2000;50:971–8.
28 Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* 2006;47:241–59.
29 Kang DJ, Ridlon JM, Moore DR, *et al*. Clostridium scindens baiCD and baiH genes encode stereo-specific 7alpha/7beta-hydroxy-3-oxo-delta4-cholenoic acid oxidoreductases. *Biochim Biophys Acta* 2008;1781:16–25.
30 Sorg JA, Sonenshein AL. Inhibiting the initiation of Clostridium difficile spore germination using analogs of chenodeoxycholic acid, a bile acid. *J Bacteriol* 2010;192:4983–90.
31 Heeg D, Burns DA, Cartman ST, *et al*. Spores of Clostridium difficile clinical isolates display a diverse germination response to bile salts. *PLoS One* 2012;7:e32381.
32 Wilson KH. Efficiency of various bile salt preparations for stimulation of Clostridium difficile spore germination. *J Clin Microbiol* 1983;18:1017–19.
33 Francis MB, Allen CA, Shrestha R, *et al*. Bile acid recognition by the Clostridium difficile germinant receptor, CspC, is important for establishing infection. *PLoS Pathog* 2013;9:e1003356.
34 Monaghan T, Mullish BH, Patterson J, *et al*. Effective fecal microbiota transplantation for recurrent Clostridioides difficile infection in humans is associated with increased signalling in the bile acid-farnesoid X receptor-fibroblast growth factor pathway. *Gut Microbes* 2018;1:1–7.
35 Friedman ES, Li Y, Shen TD, *et al*. FXR-Dependent Modulation of the Human Small Intestinal Microbiome by the Bile Acid Derivative Obeticholic Acid. *Gastroenterology* 2018;155:1741–52.
36 Swann JR, Want EJ, Geier FM, *et al*. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc Natl Acad Sci U S A* 2011;108:4523–30.
37 Theriot CM, Koenigsknecht MJ, Carlson PE, *et al*. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. *Nat Commun* 2014;5:ncomms4114.
38 Theriot CM, Bowman AA, Young VB. Antibiotic-Induced Alterations of the Gut Microbiota Alter Secondary Bile Acid Production and Allow for *Clostridium difficile* Spore Germination and Outgrowth in the Large Intestine. *mSphere* 2016;1.
39 Weingarden AR, Chen C, Bobr A, *et al*. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. *Am J Physiol Gastrointest Liver Physiol* 2014;306:G310–9.
40 Brown JR, Flemer B, Joyce SA, *et al*. Changes in microbiota composition, bile and fatty acid metabolism, in successful faecal microbiota transplantation for Clostridioides difficile infection. *BMC Gastroenterol* 2018;18:131.
41 Weingarden AR, Dosa PI, DeWinter E, *et al*. Changes in Colonic Bile Acid Composition following Fecal Microbiota Transplantation Are Sufficient to Control Clostridium difficile Germination and Growth. *PLoS One* 2016;11:e0147210.
42 Buffie CG, Bucci V, Stein RR, *et al*. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 2015;517:205–8.
43 Studer N, Desharnais L, Beutler M, *et al*. Functional Intestinal Bile Acid 7α-Dehydroxylation by *Clostridium scindens* Associated with Protection from *Clostridium difficile* Infection in a Gnotobiotic Mouse Model. *Front Cell Infect Microbiol* 2016;6:191.
44 Lawley TD, Clare S, Walker AW, *et al*. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. *PLoS Pathog* 2012;8:e1002995.
45 Solbach P, Chhatwal P, Woltemate S, *et al*. BaiCD gene cluster abundance is negatively correlated with Clostridium difficile infection. *PLoS One* 2018;13:e0196977.
46 Amrane S, Bachar D, Lagier JC, *et al*. Clostridium scindens Is Present in the Gut Microbiota during Clostridium difficile Infection: a Metagenomic and Culturomic Analysis. *J Clin Microbiol* 2018;56:e01663-17.
47 Fuentes S, van Nood E, Tims S, *et al*. Reset of a critically disturbed microbial ecosystem: faecal transplant in recurrent Clostridium difficile infection. *ISME J* 2014;8:1621–33.
48 Jalanka J, Mattila E, Jouhten H, *et al*. Long-term effects on luminal and mucosal microbiota and commonly acquired taxa in faecal microbiota transplantation for recurrent Clostridium difficile infection. *BMC Med* 2016;14:155.

Gut: first published as 10.1136/gutjnl-2018-317842 on 11 February 2019. Downloaded from http://gut.bmj.com/ on 13 June 2019 by guest. Protected by copyright.