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Roles of the gut virome and mycobiome in faecal microbiota transplantation

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4

26 **Summary**

27 Faecal microbiota transplantation (FMT) is an innovative approach to treat
28 diseases associated with a gut dysbiosis, by transferring a healthy stool
29 microbiota to a diseased recipient. Beyond the bacteriome, the human gut
30 also harbours diverse communities of viruses and fungi, collectively known
31 as the virome and the mycobiome. The impact of these latter two microbiome
32 components on success of FMT therapy has not been appreciated until very
33 recently. We herein review the current literature on the effects of the gut
34 virome and mycobiome in the FMT treatment of various diseases. We
35 discuss both the beneficial effects and health concerns of the viral and fungal
36 transfer during FMT. We particularly highlight the roles of bacteriophages
37 (bacterial viruses) and *Candida* species (fungi) in FMT efficacy. We also
38 summarise the intricate relationships between the gut virome, mycobiome,
39 bacteriome, and host immunity, underlying FMT. Future efforts should be
40 devoted to understanding the versatile roles as well as the therapeutic
41 mechanisms of specific and/or combination of viral and fungal lineages in
42 different diseases. Harnessing the gut virome, mycobiome, and bacteriome in
43 combination and precision hold a promising prospect in future FMT- and
44 microbiota-based therapies.

45

Introduction

A large number of diseases are characterized by compositional and functional changes in the gut microbiota. Faecal microbiota transplantation (FMT) is a gut microbiota restoration treatment, performed through oral, intra-intestinal or intra-colonic administration of donor faecal matter containing natural microbial consortia. It is well established that the bacterial microbiome (bacteriome) plays a prominent role in the pathogenesis of gastrointestinal tract (GI) diseases and affects the outcome of therapies.[1,2] Apart from bacteriome, the human gut contains diverse and largely under-explored communities of viruses and fungi.[3,4] Recent evidence suggests that the gut virome and mycobiome not only constitute a significant fraction of the total microbiome, but also work in synergy with the bacteriome to modulate host immunity and physiology.[5,6] Evidence on the roles of gut virome and mycobiome in FMT outcome is also accumulating.[7–9]

FMT has garnered substantial clinical and translational research interests.[10,11] It has broad applications across different diseases, both intra- and extra-intestinal diseases, including *Clostridioides difficile* infection (CDI), recurrent CDI (rCDI), inflammatory bowel diseases (IBD), graft versus host disease (GvHD), irritable bowel syndrome (IBS), obesity and diabetes.[8–10,12–14] While FMT was previously found comparable with the treatment of probiotics in efficacy when treating various diseases, it was recently demonstrated that autologous FMT, but not a multi-strain probiotic product, was effective in post-antibiotic restoration of microbiome in mice.[15,16] This is likely due to the fact that FMT transfers a complete and complex consortium of host-adapted microbial species, including bacteria, bacteriophages, fungi, as well as their metabolites, as opposed to a greatly simplified multi-strain bacteriotherapy. The efficacy of FMT has long been ascribed to the transfer of bacteria.[10,11] Compared to that, the roles of virome and mycobiome are relatively less studied and are yet to be fully emphasized.[2,6,17–19]

77 In this review, we aim to summarise relevant clinical, translational, and basic
78 research evidence in the field to enhance the community's understanding of
79 the roles of gut virome and mycobiome in FMT, to inform better clinical
80 practice by incorporating the viral and fungal components of the gut
81 microbiome into FMT regime, and in a broader context, to guide future
82 development of microbiota-based therapies. We will discuss the roles of gut
83 virome and mycobiome in FMT in conjunction with their functional
84 importance, donor-recipient effect, as well as safety concerns, and highlight
85 how they potentially impact treatment efficacy and host immunity during FMT.

86

87 Roles of gut virome in FMT

88 The human gut virome

89 The human gut virome is primarily comprised of prokaryotic viruses (mostly
90 bacteriophages infecting bacteria, collectively known as 'phageome') and
91 eukaryotic viruses (infecting humans), with bacteriophages of the order
92 *Caudovirales* (tailed icosahedral viruses with dsDNA genomes) and the
93 family *Microviridae* (small icosahedral viruses with ssDNA genomes) being
94 most abundant (figure 1a).[3,20–22] Bacteriophages in the human GI tract have
95 both temperate and lytic lifecycles, driving the bacteriome composition by
96 prophage integration and lytic predation respectively.[3,23] Healthy human gut
97 virome is dominated by temperate bacteriophages, while it shifts from
98 temperate to lytic replication during host inflammation and stress.[3,24] Given
99 the predominance of bacteriophages over eukaryotic viruses in the gut
100 virome and its direct roles in regulating bacteriome composition and function,
101 most gut virome research in humans has been focusing on the phageome.
102 While a minority of eukaryotic viruses can cause serious infections, emerging
103 data on a large diversity of gut-resident eukaryotic viruses show that they are
104 somewhat capable of recapitulating the beneficial effects of commensal
105 bacteria through different mechanisms involving host immunity.[25,26]
106 Eukaryotic viruses can ameliorate gut inflammation in mice via viral RNA
107 sensing by host Toll-like Receptors (TLRs)-3 and 7 and its downstream IFN-
108 β secretion, and antiviral treatment led to more severe colitis in dextran

109 sulfate sodium (DSS)-treated mice.[27] A recent study in mice also shows that

110 enteric eukaryotic viruses evoke broad and enduring host immune responses

111 resembling those elicited by the commensal bacteria.[28] These data suggest

112 that gut viruses play an important role in host immunity and homeostasis.

113

114 Gut viruses overall constitute a more diverse genetic entity than the gut

115 bacteria, with virus to microbe ratio (VMR) ranging from 1:1 to 10:1 in the

116 gut.[29,30] Human gut virome is highly diverse and immensely affected by

117 geography, ethnicity, diet, lifestyle and age (figure 1a, together accounting for

118 ~30% of gut virome variations).[31,32] Gut virome dysbiosis has been implicated

119 in the pathogenesis of a diversity of GI and extra-GI diseases, such as

120 IBD[21,33], IBS[34], CDI[7,35], GvHD[12,36], obesity and diabetes[37,38], and FMT have

121 demonstrated a varying degree of success in treating these diseases.[7,12,14,39–]

122 [42] One commonly observed feature of the gut virome alterations in GI

123 inflammation-related diseases, including in CDI and IBD, is expansion of

124 *Caudovirales*, which was significantly decreased after FMT.[7,21,33,35,43]. In a

125 DSS-induced colitis model of a mouse colony without colonisation of *E. coli*,

126 cocktail of *Enterobacteriaceae* bacteriophages, belonging to *Caudovirales*,

127 exacerbated intestinal inflammation and did not induce lysis of any

128 endogenous microbes.[43] This study corroborated that *Enterobacteriaceae*

129 phages alone were sufficient to elicit inflammatory responses without

130 engagement of *Enterobacteriaceae* or its constituent LPS. These data

131 highlight the importance of *Caudovirales* bacteriophages in gut homeostasis

132 and inflammation and that *Caudovirales* can be manipulated by FMT.

133

134

135 **Translational studies investigating FMT and virome**

136 Transfer and engraftment of viruses, along with bacteria, during naïve FMT

137 correlated with treatment outcome in difference diseases, including CDI, IBD,

138 GvHD following hematopoietic stem cell transplant (HSCT).[7,12,44–47] In a

139 seminal study conducted by Ott et al, the authors showed that sterile faecal

140 filtrate (containing viruses, as well as various metabolites and polymers, but

141 not bacteria) was sufficient for successful remission in patients with CDI[48].
142 Following that, a number of studies further emphasised the importance of gut
143 viruses, beyond bacteria, in FMT therapies.[7,14,48,49] In recent years, a handful
144 of sterile faecal filtrate and faecal viral transplantation (FVT) studies have
145 emerged investigating the role of gut virome in disease therapeutics,
146 including obesity, type 2 diabetes (T2D), necrotizing enterocolitis (NEC),
147 small intestinal bacterial growth, and post-antibiotic microbiome
148 dysbiosis.[14,49–51]

149

150 ***FMT in CDI***
151 The efficacy of FMT in treating CDI is 90%, which is the most promising
152 compared to its applications in other diseases.[52–56] In our pilot FMT study on
153 patients with CDI (n=9; FMT was conducted via nasoduodenal infusion of
154 donor stool from a healthy household individual to each patient), we found
155 that the gut virome of CDI was characterized by a significant elevation in
156 *Caudovirales* bacteriophages and a reduced virome diversity compared to
157 healthy individuals.[7] After FMT, patients showed substantial viral
158 transmission from donor to recipient.[7] Patients who were cured from CDI
159 exhibited much higher engraftment rates of *Caudovirales* taxa derived from
160 the FMT donor, than those who were not responsive to FMT.[7] Recently, a
161 study on rCDI (n=9) showed that after FMT, the coding genes repertoire of
162 the patients' gut virome was more similar to that of the donors' compared to
163 their pre-FMT profiles, including KO (Kyoto encyclopedia genes and
164 genomes Orthology) terms associated with viral replication, iron transporters,
165 ssDNA phage assembly, and antimicrobial peptide resistance, paralleling the
166 changes in their host bacteria after FMT.[35] This data indicates a potential
167 functionality change in the gut virome of recipient along with the
168 compositional changes in viral taxa after FMT. Concordantly, several studies
169 reported that recipient viromes resembled those of their donors and remained
170 stable after FMT.[7,46,47] Among these studies, one with longer follow-up
171 showed that patients with CDI (n=14) showed a post-FMT gut virome profile
172 similar to that of their donors for up to 1 year after FMT.[47]

173

174 ***FMT in IBD***

175 The efficacy of FMT in treating IBD varies from ulcerative colitis (UC) to

176 Crohn's disease (CD). A systematic analysis documented 53 studies that the

177 overall FMT efficacy is around 36% in UC, 50.5% in CD and 21.5% in

178 patients with pouchitis.[57] In patients with IBD, the faecal virome displayed

179 increased richness of *Caudovirales* bacteriophages along with a decreased

180 bacterial diversity (n=174; patients were from Cambridge, UK; Chicago, Los

181 Angeles, and Boston, in USA).[21] At the intestinal mucosal level, patients with

182 UC (n=91; three China cohorts) showed that the rectal tissues consistently

183 had an expansion of *Caudovirales* bacteriophages compared with healthy

184 individuals. [33] Phages of *Escherichia* and *Enterobacteria*, belonging to the

185 order *Caudovirales*, were experimentally demonstrated to aggravate

186 intestinal inflammation and colitis as a consequence of overproduction of

187 IFN-γ via TLR-9 signalling in the murine host [43]. These data together suggest

188 that certain *Caudovirales* taxa play a crucial role in the disease course of

189 IBD. However, the role of the phageome (particularly *Caudovirales*

190 bacteriophages) in FMT therapies of IBD has not been thoroughly

191 investigated to date, which warrants in-depth research.

192

193 An increased eukaryotic virome richness was also observed in faeces of

194 patients with UC.[44] One pilot FMT study in UC patients (n=9) found that UC

195 individuals who successfully responded to FMT contained a significantly

196 lower eukaryotic virome richness (both before and after FMT) compared to

197 non-responders.[44] This study indicates that a low baseline eukaryotic viral

198 richness might be important for a successful FMT in UC, albeit the

199 mechanism is lacking. Overall, the role of viral engraftment and virome

200 alterations in FMT therapies targeting IBD is still in its infancy and represents

201 an area of particular interest.

202

FMT in GvHD

Limited studies have found some effectiveness of FMT in treating GvHD, though the overall efficacy is lacking.[12,58] Patients with GvHD following HSCT manifested significant GI symptoms and showed a persistent dominance of eukaryotic viruses (anelloviruses, herpesviruses, papillomaviruses and polyomaviruses) in the gut.[36] Among them, picobirnaviruses were determined as a predictive marker for the development of severe GvHD.[36] In a single case study, a GvHD patient was treated with 4 episodes of FMT. [12] Following treatment, an increase in faecal virome diversity was observed, accompanied by expansion of *Caudovirales* bacteriophages and shrinkage in the eukaryotic Torque teno viruses.[12] Consistent with FMT effects on virome reported in CDI, the transfer of *Caudovirales* bacteriophages also underpins a positive treatment outcome in GvHD.[7,12] Future research with expanded sample sizes is needed to convincingly address the therapeutic effect of FMT and virome modulations in GvHD.[58,59]

Faecal viral transplantation (FVT) in other disease indications

As viruses are co-transferred alongside bacteria during naïve FMT, the role of viruses is fastidious to tease apart from that of bacteria in treating disease. Two pilot case series studies (n=5 and 4) explored the effect of sterile faecal filtrate in treating CDI, a refined FMT protocol where the bacteria were filtered out.[48,60] This study showed that sterile faecal filtrate restored normal stool habits and eliminated symptoms of CDI, suggesting a possible role of bacteriophages and viruses (as a prominent component of sterile faecal filtrate) in the therapeutic mechanism of FMT. However, caution should be taken when interpreting these studies considering that the biological effect of sterile faecal filtrate is not solely attributed to the viral microbiota *per se*, where the metabolites, digested micro-nutrients, extracellular active ingredients (such as viral envelope, bacterial cell wall components and antimicrobial peptides) are also present in sterile faecal filtrate.

234 Following that, more studies have employed a further refined protocol, FVT,

235 to specifically investigate the effect of faecal viruses in treating diseases,

236 where faecal virus-like particle (VLP) are isolated, purified, and enriched.[14,49–

237 51] In a piglet model of preterm infants, Brunse et al. showed that orally

238 administrated FVT successfully prevented the development of necrotising

239 enterocolitis (NEC) in all preterm piglets that received FVT, whereas FMT did

240 not perform better than the control arm, indicating that a gut virome transfer

241 and modification might be critical in preventing the development of NEC.[50]

242 Following antibiotic-mediated disruption of the bacteriome in human patients,

243 rapid restoration of the depleted microbiota might confer significant health

244 benefits.[49] Probiotics were shown to be not only ineffective in post-antibiotic

245 restoration of the gut microbiome, but sometimes even leading to impaired

246 microbiome recovery[15,16]. In contrast to that, a study investigated the effect of

247 autologous FVT (harvested prior to antibiotics perturbation) in mice reported

248 a better gut bacteriome recovery after antibiotic perturbation, compared to

249 the control treatment.[49] More recently, a study reported the effect of FVT in

250 treatment of type 2 diabetes and obesity in mice.[14] Obese mice treated with

251 FVT showed improvement in glucose tolerance and reduced further weight

252 gain caused by high-fat diet.[14] In these mice, FVT was found to impact not

253 only the virome but also the bacteriome, due to the transfer of

254 bacteriophages.[14] In addition, FVT can reduce high-fat diet-induced small

255 intestinal bacterial overgrowth.[51] Taken together, these findings emphasise

256 the critical role of virome transfer in successful FMT therapies of various

257 human diseases.

258

Donor and recipient effect

260 In our prior FMT-CDI study, we found that donor-recipient pairs with a higher

261 faecal viral richness of *Caudovirales* in donor than recipient was predictive of

262 a better clinical outcome in CDI.[7] This result at the same time was coupled

263 with changes in the bacteriome, where FMT-driven *Caudovirales* transfer

264 was positively correlated to the bacterial richness and diversity in the

265 responders.[7] Due to the co-transfer nature of virome and bacteriome during

266 FMT, it precludes us separating the effect of virome and that of bacteriome in

267 influencing FMT outcome in this setting. Albeit, it highlights the importance of
268 donor virome composition and significance of appropriate donor-recipient
269 pairing for the efficacy of FMT. Consistently, another study found that donors
270 with a high faecal bacteriophage α-diversity and a low bacteriophage
271 abundance were associated with a successful FMT in CDI.[45]

272

273 By adopting a single-cell viral tagging (VT) approach, researchers
274 investigated whether individual phages isolated from one subject's faeces
275 could interact with bacteria isolated from a different subject, as a proxy for
276 FMT.[61,62] They found that a high level of cross-reactivity between
277 bacteriophages and bacteria from different human subjects, which however
278 varied across donor-recipient pairs, highlighting a significant donor-recipient
279 pairing effect on FMT/FVT outcomes.[62] Therefore, by modelling a bacteria–
280 bacteriophage interaction network between the donor and recipient microbial
281 communities, based on single-cell VT results, it may become possible to
282 identify optimal donor-recipient pairs and to predict the outcomes of FMT.

283

284 **Safety concerns of virome transfer in FMT**
285 While data suggests that viral transfer during FMT is an important beneficial
286 factor for the success of therapy, undesirable viral transmissions constitute a
287 serious safety concern, especially when transplanting into an
288 immunocompromised recipient. One report showed that norovirus was
289 transmitted to 2 out of 13 CDI patients via FMT procedure, who later
290 developed a post-FMT norovirus gastroenteritis.[63] This report claimed that
291 viral contamination and transfer during the FMT procedure by a procedure-
292 involved employee who had previously developed norovirus-like symptoms
293 might be a cause to the observed post-FMT norovirus gastroenteritis.[63] A
294 number of pathogenic eukaryotic viruses can potentially be present in the
295 human gut, including papillomaviruses, herpesviruses, hepatitis viruses,
296 bocaviruses, enteroviruses, rotaviruses, and sapoviruses.[64] To prevent
297 potential transfer of pathogenic eukaryotic viruses during FMT, a thorough
298 faecal virome screening of the donor should be performed to ensure the

299 safety of faecal transplant. Despite such concerns, FVT seems to be
300 generally safer than FMT due to the removal of intact bacteria, in particular
301 obligate and opportunistic bacterial pathogens, prior to transplantation. FVT
302 can also avoid transfer of certain gut commensals with unwanted properties,
303 which under certain conditions can interact with host physiology exacerbating
304 disease. Overall, FMT in general has been found to be safe provided that the
305 donor screening is done adequately as instructed in international guidelines.
306 Most, if not all, unfortunate cases have been due to sloppy and highly
307 unacceptable donor screening.

308

309 Bacteriophages targeting gut bacteria can have cascade effects on bystander
310 bacteria, as demonstrated in gnotobiotic mice.[65,66] Therefore, which gut
311 bacteria are going to be affected by phage predation during FMT, to what
312 extent and in what direction, present an unpredictable and daunting
313 challenge. Equally complex and unpredictable is the subsequent effects of
314 microbiome manipulations on the overall health of the human host. In
315 addition, horizontal gene transfer (HGT) from one bacterial strain to another,
316 mediated by bacteriophages (phage transduction) can be a significant
317 contributor to dissemination of antibiotic resistance genes, virulence genes
318 and other unwanted genetic material.[67] . A number of bacteriophages encode
319 toxins (such as diphtheria toxin, Shiga toxin and erythrogenic toxin) that
320 increase survival and virulence of their bacterial host during lysogenic
321 conversion.[68,69] FMT may transfer the bacteriophages carrying these toxin
322 genes to confer a virulent phenotype to the co-resident bacteriome in the gut
323 of the recipient, posing another health concern.

324

325 Administering rats with a bacteriophage cocktail led to an increased intestinal
326 permeability, weight loss, and decreased activity.[70] This study demonstrates
327 that increased intestinal permeability may be induced by bacteriophages that
328 affect the microbiota. Recently, a temperate filamentous bacteriophage was
329 found to integrate into the genome of a multidrug-resistant *Pseudomonas*
330 *aeruginosa*, resulting in inappropriate antiviral immune responses and

331   impaired clearance of bacterial infection in the host.[71] These evidence
332   suggest that akin to the transfer of certain eukaryotic viruses, the transfer of
333   unwanted bacteriophages may also confer undesired health concerns to the
334   human host.

335

336   **Mechanisms of virome function underlying FMT**
337   The mechanisms of viral action contributing to FMT therapies include
338   tripartite mutualistic interactions between bacteriophages/eukaryotic viruses,
339   bacteria, and the mammalian host (figure 1b).[6]

340

341   ***Functions of bacteriophages colonisation***
342   Bacteriophage transfer engraftment can modulate not only the taxonomic
343   composition but also the functional capacity of the gut bacteriome.[65,66,72]
344   Faecal multi-omics profiling of mice receiving a transplant of a defined
345   consortium of bacteriophages revealed shifts in both the microbiome and gut
346   metabolome after bacteriophage colonisation.[66] The result showed that a
347   large diversity of metabolites was altered after bacteriophage transplantation,
348   including nearly all KEGG pathways (amino acids, peptides, carbohydrates,
349   lipids, nucleotides, cofactors, vitamins, and xenobiotics).[66] A recent study
350   also showed that Bacteroides phage BV01 altered the genome-wide
351   transcriptome profiles and bile salt hydrolase activity in its bacterial host,
352   leading to an altered profile of bile acids.[72] In return, bile acids are well known
353   to regulate both host microbiome (including *C. difficile*) and host
354   physiology.[73,74] Such changes in gut bacteriome composition and functionality
355   induced by bacteriophage colonisation are important for host health.

356

357   Accumulating data suggest that bacteriophages can modulate the immune
358   system both directly and indirectly. Indeed, bacteriophages can colonise the
359   intestinal mucus layer, directly binding to mucin glycoproteins via their
360   capsids, and provide the mammalian host with a defence mechanism against
361   the bacteria trying to breach through the intestinal barrier.[75] Certain

362 bacteriophages, such as phage 536_P1, directly promote the production of
363 antiviral cytokines, such as IFN-γ and IL-12, as well as chemokines, even in
364 the absence of their host bacteria.[76] Bacteriophages can interact with the
365 host immune system in various ways, by inducing the innate defenses
366 against bacterial colonisation, stimulating production of inflammatory
367 cytokines, and activating dendritic cells (DCs) and innate lymphoid cells
368 (ILCs) to produce IFNs (figure 1b).[77]

369

370 ***Functions of eukaryotic viruses colonisation***
371 In addition, colonisation with eukaryotic viruses in the gut is critical for the
372 maintenance of gut microbial homeostasis and host immunity. Recognition of
373 the bulk of enteric viruses via surface receptors TLR3 or TLR7 induces
374 production of IFN-β, which protects the host from developing inflammation.[27]
375 The RIG-I receptor for viral RNA recognition in the cytosol signals IL-15
376 production to maintain homeostasis of intraepithelial lymphocytes.[78] Another
377 prime example of protective effects of intestinal eukaryotic virome was
378 demonstrated in murine norovirus (MNV)-colonised mice.[79] The viral protein
379 NS1/2 from MNV evoked a host protective response with increased
380 production of IFN-I and IL-22, which conferred a critical protection against
381 *Citrobacter rodentium* infection and promoted proliferation of the intestinal
382 epithelial cells.[79] A recent comprehensive murine study profiled the immune
383 responses to a panel of eukaryotic viruses and found a widespread capacity
384 for asymptomatic intestinal colonisation and durable alterations that the both
385 strain-specific and common to multiple viruses.[28] Most enteric viruses
386 promoted T cell differentiation, Th1 polarization, and production of IL-22, a
387 cytokine central to the dialog between host and microbiome at epithelial
388 barriers.[28,80] Such enteric viruses could transcriptionally upregulate anti-
389 bacterial peptides, though to a lesser extent than a consortium of 15 bacterial
390 strains in germ-free (GF) mice.[28] In addition, astrovirus supplementation in
391 immunocompromised mice can protect the mice from enteric pathogens via
392 IFN-λ, transferable by cohousing and faecal transplantation.[81] These data
393 together suggest that gut eukaryotic viruses also tune host homeostasis by
394 orchestrating both the host immunity and the co-resident microbiome.

395

396 Taken together, these studies indicate that colonisation by gut viruses, both
397 prokaryotic and eukaryotic, is essential to calibrate host immunity and
398 physiology. Transfer and engraftment of gut viruses via FMT can have broad
399 cascading effects on the mammalian host, including modulation of host
400 microbiome, metabolome, and immunity.

401

402 Roles of gut mycobiome in FMT

403 The human gut mycobiome

404 The human GI tract is also home to a large diversity of fungi, collectively
405 known as the gut mycobiome. Fungi constitute a minor component of the gut
406 microbiota, making up approximately 0.1% of the microorganisms shown by
407 metagenomics sequencing.[4,82,83] Studies have shown that the gut harbours
408 >50 genera of fungi with *Candida*, *Saccharomyces*, *Cladosporium* and
409 *Malassezia* being the most abundant genera (figure 2a).[4,82,83] However, the
410 gut fungi are highly under-explored relative to the gut bacteria, and hence are
411 underrepresented in current mycobiome databases, hindering gut
412 mycobiome profiling and functional characterisation.[84] Gut resident fungi
413 have both mutualistic and antagonistic relationships with the gut bacteria,
414 together shaping the host immunity.[85] Intestinal fungi have been shown to be
415 causally implicated in microbiome assembly and immune development.[86]
416 Accumulating evidence points to that the gut mycobiota can strongly
417 influence the host immune system and this interaction is linked to bacteria
418 activities.[87,88] Recent observations of dysbiosis in gut mycobiome across
419 various diseases highlight a critical role of gut fungi in disease
420 pathogenesis[89–91], suggesting that targeting gut mycobiome may represent a
421 promising therapeutic modality.

422

423 Translational studies investigating FMT and mycobiome

424 Emerging studies report that FMT involves transfer of fungi, which may affect
425 treatment outcomes.[8,9] Our FMT-fungi study in CDI (n=16) showed that donor

426 fungi were substantially transferred to recipient after FMT, and cure after
427 FMT was associated with increased colonisation of donor-derived fungal taxa
428 in recipients.[8] After FMT, responders displayed a high relative abundance of
429 *Saccharomyces* and *Aspergillus*, whereas non-responders and patients
430 treated with antibiotics displayed a dominant presence of *Candida* in faeces.[8]
431 CDI patients who responded to the treatment showed a reduction in the
432 abundance of *C. albicans* after FMT, while those who did not respond to FMT
433 showed a still high abundance of *C. albicans* in faeces.[8] In favor of this
434 finding in humans, we observed that *C. albicans* also negated FMT efficacy
435 in a mouse model of CDI.[8] In a separate experiment, we showed that
436 antifungal treatment to eliminate *C. albicans* in recipient mice before FMT re-
437 established FMT efficacy using the same donor stool without presence of *C.*
438 *albicans*.[8] These evidence together highlight a causal relationship between
439 gut fungal dysbiosis and FMT outcome in CDI.

440

441 Interestingly, a recent FMT study conducted in patients with UC (n=24)
442 observed a different association between faecal *C. albicans* levels and
443 treatment outcome: UC patients with higher *C. albicans* abundance pre-FMT
444 were much likely responsive to FMT. [9] On the other hand, in agreement with
445 the finding in CDI, FMT resulted in a reduction of *C. albicans* in UC patients.[9]
446 Decreased *Candida* abundance post-FMT was indicative of ameliorated UC
447 severity. [9] These findings suggest that FMT reduces *C. albicans* abundance,
448 and a decreased *C. albicans* abundance after FMT is associated with
449 disease amelioration in both CDI and UC. The discordant findings between
450 CDI and UC that a high faecal abundance of *C. albicans* in recipients pre-
451 FMT may lead to discrepant treatment outcomes after FMT underscores that
452 gut fungi in recipient baseline may affect FMT efficacy in a disease-
453 dependent manner. Similarly, patients with CD had higher fungal burden and
454 *Candida* colonisation in the gut, however CD patients were less responsive to
455 FMT compared to UC.[10,92–94] Different immunophysiology and
456 immunopathophysiology mechanisms, though calibrated by the same fungi,
457 in different disease settings may underlie this discordance in FMT efficacy
458 between diseases.

459

460 Overall, FMT treatment is able to reduce *C. albicans* levels in recipient and
461 produce a positive treatment outcome in CDI and IBD. This effect is also
462 evidenced in different mouse models, demonstrating that FMT prevented *C.*
463 *albicans* colonisation.[8,95,96] In a pilot study, a patient with UC was cleared of
464 recurrent *C. glabrata* infection after FMT [97], suggesting FMT may be able to
465 deliver a possible beneficial effect on patients who are complicated with
466 fungal infection. Another clinical study showed that FMT was effective to
467 reduce the likelihood of developing blood stream fungal infection in rCDI
468 patients compared to antibiotics treatment.[98] These studies further support
469 that FMT is capable of modulating recipient fungi, including *Candida* species.

470

471 Another intestinal disorder IBS is also characterised by a significant alteration
472 in the gut mycobiome, featured by predominance of two fungal species *C.*
473 *albicans* and *Saccharomyces cerevisiae* compared to healthy individuals.[99]
474 Interestingly, IBS-related visceral hypersensitivity was transferable between
475 rats by FMT, suggesting a causal role of gut microbiome in IBS.[99] To date,
476 whether FMT is effective in IBS is still controversial in clinical practices. One
477 randomized clinical trial (n=90) conducted in a cohort of IBS patients in
478 northern Norway showed that FMT relieved IBS symptoms compared to the
479 placebo arm, whereas another randomized clinical trial (n=48) conducted in
480 three US centres showed that FMT was ineffective in reducing IBS symptoms
481 compared with placebo.[100] Whether gut fungi play a role in such
482 heterogenous clinical efficacy of FMT in IBS warrants further investigation.

483

484 FMT is a promising agent for treating infectious diseases and gastrointestinal
485 disorders, it has been utilised for treating severe colitis in GvHD patients
486 following allogenic HSCT.[12] The antifungal drug fluconazole has been shown
487 to prevent *C. albicans* colonisation and to reduce the likelihood of developing
488 GvHD after HSCT, suggesting that modulation of gut mycobiota may be
489 associated with the pathogenesis of GvHD post HSCT.[101] More recently, our
490 observational FMT study on a single GvHD case reported some changes in

491 the gut mycobiome following a successful treatment of 4-dose FMT.[12] The
492 serial FMT treatments altered the fungal composition and diversity in the
493 faeces of this GvHD patient, which was more similar to the donor's faecal
494 fungal profile after FMT compared to his pre-FMT profile.[12] However, as this
495 pilot study was exploratory, an expanded sample size is needed in future to
496 address any biologically meaningful changes in recipient's gut mycobiome by
497 FMT underlying cure of GvHD.

498

499 The role of gut mycobiome in FMT is elusive and there is a significant lack of
500 human and animal studies centered on fungal transfer in FMT treating
501 different diseases. The keystone fungal species as well as their functions in
502 the gut and FMT remain to be discovered in different disease settings.

503

504 **Donor and recipient effect**
505 As aforementioned, our FMT-CDI study in both humans and mice found that
506 existence of *C. albicans* in donor and high abundance existence of *C.*
507 *albicans* in CDI recipient pre-FMT were both detrimental to FMT outcome.[8]
508 Similarly, two filamentous fungi *Penicillium brocae* and *Aspergillus*
509 *penicillioides*, present in either donor or recipient pre-FMT, also nullified the
510 treatment efficacy of FMT in CDI mice, highlighting a generic deleterious role
511 of overrepresentation of certain fungi in donor or recipient in FMT.[8] Antibiotics
512 are always the primary treatment for patients with CDI, which may lead to a
513 fungal bloom and a high likelihood of developing fungal infections in patients,
514 particularly expansion of *Candida* species.[102] These data underscores the
515 importance of donor selection (exclusion of invasive *Candida* carriers) and
516 appropriate recipient preparation based on their faecal fungal profiles, to
517 enhance FMT efficacy in CDI. In contrast, a high faecal abundance of *C.*
518 *albicans* in UC patients pre-FMT was associated with a favourable FMT
519 outcome.[9] Taken together, it suggests that the gut mycobiome composition of
520 donor and recipient should be considered separately in different disease
521 settings when employing FMT.

522

Safety concerns of mycobiome transfer in FMT

Analogous to gut bacteria, a lot of gut fungi are opportunistic species which can mount detrimental immune responses in the host under non-homeostatic conditions, including *C. albicans.* Therefore, to avoid transfer of fungal pathogens and opportunistic fungal pathogens, thorough donor screening based on the faecal fungal profile is necessary during FMT practice. To date, there has no report of death or infection caused by transmission of life-threatening fungi from FMT, but screening for fungal candidates in the donor and susceptible recipients should also be cautious. Fungal infection is often seen in immunocompromised patients with CDI, IBD and GvHD, and the responsible candidates are *Candida* species that contribute to the majority of infections.[97,98,101] Most common fungal infections in CDI are caused by *C. albicans*, *C. parapsilosis*, and *C. tropicalis* in the blood stream due to prolonged use of antibiotics.[98] These fungi constitute a health concern to the host, and may nullify FMT efficacy and invoke undesired immune responses after FMT.

Other fungi, such as *Malassezia restricta* and *Histoplasma capsulatum* should also be checked in donor screening.[103,104] *M. restricta* is a skin commensal that preferentially colonises in CD patients with CARD9 polymorphism, whereas *H. capsulatum* preferentially infects IBD patients with immunocompromised condition. [103,104] CARD9 and dectin-1 are well known fungal recognition receptors, and polymorphisms in these genetic loci are associated with increasing susceptibility to fungal infections.[105–107] It is known that antibiotics can alter the gut bacterial-fungal community structure and that antibiotics treatment was found to offer longer-lasting impact on gut fungi other than bacteria.[108] Hence, fungal profiling in the donor, the genetically susceptible or immunosuppressive recipient, as well as those with a drug or antibiotics history, are needed to avoid potential fungal bloom or infection following FMT. [105–107,109]

Mechanisms of protective immunity elicited by the gut fungi

The commensal fungi in the gut can evoke protective immunity in the host and impact gut microbiome assembly. [6,88,105,110,111] The mechanisms of mycobiome action underlying FMT treatment of diseases hence also involve a tripartite interaction between fungi, bacteria, and the mammalian host (figure 2b).

It is shown in gnotobiotic mice that commensal fungi can functionally recapitulate the protective benefits of intestinal commensal bacteria, by mitigating tissue injury and extra-intestinal infection as well as calibrating the activation of protective CD8+ T cells.[112] Elimination of the gut fungi by oral antifungals in mice worsened the outcome of colitis and allergic airway disease[91], where both the host immune profile and the gut bacteriome composition were disrupted, suggesting that colonisation of gut commensal fungi plays an import role in host immune and microbiome homeostasis. Fungi can stimulate host cells through a variety of microbial pattern recognition receptors (PRRs). [6,105,110,113–117] Surface receptors, such as dectin-1, dectin-2, mincle and CX3CR1 receptors on mononuclear phagocytes (MNPs) can recognize β-glucans in the fungal cell wall.[6,105,110,113–117] Fungal sensing by CARD9 signalling induces release of IL-18 for reconstitution of intestinal epithelial cells (IECs).[6,105,110,113,118] IL-22 elicited by fungal sensing restricts inflammasome from triggering immune pathology.[119] Fungal-derived molecules and metabolites, such as mannans and glucans, are also critical components to calibrate host immunity by changing the cytokine profile.[120,121] These mechanistic studies underscore the sophisticated molecular pathways underlying gut fungi regulating host immunity.

Another aspect of mycobiome function on the host is its interactions with gut bacteria and other co-resident fungi. In steady state, bacteria and fungi keep each other in check in the gut. One study demonstrated that *C. albicans* affects the recolonisation of the cecum by the microbiota in mice treated with antibiotics.[122] The presence of *C. albicans* in the gut increased colonisation

586 by the bacterium *Enterococcus faecalis* and reduced colonisation of probiotic

587 *Lactobacillus* strains.[122] A follow-up study revealed that antibiotic-treated *C.*

588 *albicans*-colonised mice had reduced expression of specific immune genes,

589 hinting at dual role of *C. albicans* on host immunity and bacteriome

590 assembly.[123] Several studies have shown that *Saccharomyces boulardii* can

591 suppress *C. difficile* by the production of a protease to degrade toxins A and

592 B of *C. difficile*.[124,125] These studies imply that colonisation of *Saccharomyces*

593 species by FMT may favour a positive outcome in CDI. Moreover, *S.*

594 *boulardii* has a protective effect against various other bacterial

595 gastrointestinal pathogens, including *Helicobacter pylori*, *Vibrio cholerae*,

596 *Salmonella enterica serovar Typhimurium*, *Shigella flexneri*, and *Escherichia*

597 *coli*.[85] Both *E. coli* and *S. Typhimurium* bind to the surface of *S. boulardii*,

598 potentially preventing adhesion to intestinal epithelial cells and thus allowing

599 quicker excretion through faecal matter.[126,127] *S. boulardii* was found to

600 suppress colonisations of both *C. albicans* and Adherent-invasive

601 *Escherichia coli* (AIEC) and to alleviate colitis in mice.[128–130] Overall, FMT is

602 able to cause a collection of fungi colonised in recipient, where they

603 individually or together with gut bacteria regulate host immnunophysiology

604 influencing FMT outcome.

605

Conclusions

607 A large body of studies demonstrated efficacy of FMT in re-shaping the

608 microbial composition and function in the recipients in a number of human

609 diseases, though resolution of symptoms and cure can only be consistently

610 achieved in CDI and inconsistently achieved in other diseases, such as IBS,

611 IBD and obesity. Successful FMT is not only ascribed to the restoration of

612 healthy gut bacteriome, but also involves modulation of the virome and

613 mycobiome. The intricate relationships between the bacteriome, the virome

614 and the mycobiome on one hand, and the human host on the other, underpin

615 clinical and microbiological effects, and overall efficacy of FMT. Enhanced

616 understanding of gut virome and mycobiome will guide and facilitate future

617 precision FMT-based therapies of various human diseases. Well-designed

618   clinical trials based on targeted bacterial-, viral/phage- and fungal-transfer will

619   be required, so are preclinical mechanistic studies investigating functions of

620   the individual components of the microbiome. Of particular interest are

621   different lineages and combinations of *Caudovirales* phages, as well as fungi

622   from the genera *Candida* and *Saccharomyces*, due to their possible

623   contributions to FMT efficacy. Precision FMT should be adopted in future,

624   including FVT and/or defined consortia of phages-fungi-bacteria

625   combinations, in a personalised, disease-specific manner. We look forward

626   with optimism to the future of precision FMT, as it transfers a holistic, tailor-

627   made, and well donor-recipient paired microbiome for disease treatment.

628

Search strategy and selection criteria

A literature search was performed on Pubmed, Google Scholar and Web of Science using the keywords "Faecal microbiota transplantation", "FMT", "Faecal viral transfer", "FVT", "Fungal microbiota", "Gut virus", "Virome", "Gut fungi" and "Mycobiome" to select relevant clinical and animal studies.

Contributors:

TZ conceived the manuscript. TZ and SL wrote the manuscript. ANS, PL and XJW provided significant intellectual contribution and edited the manuscript. TZ, PL and XJW supervised this study. HP and XWB provided critical comments.

Declaration of interests:

The authors declare no conflict of interest.

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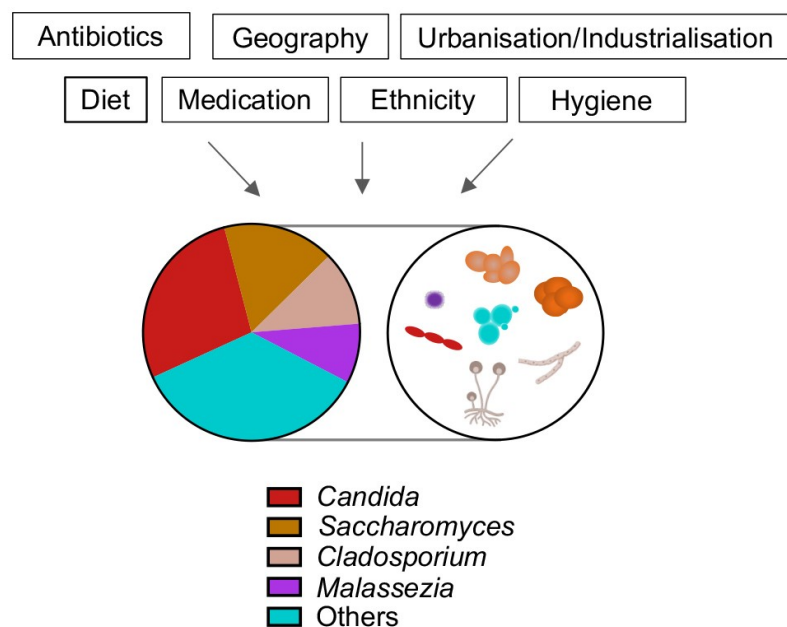1045   276–86.

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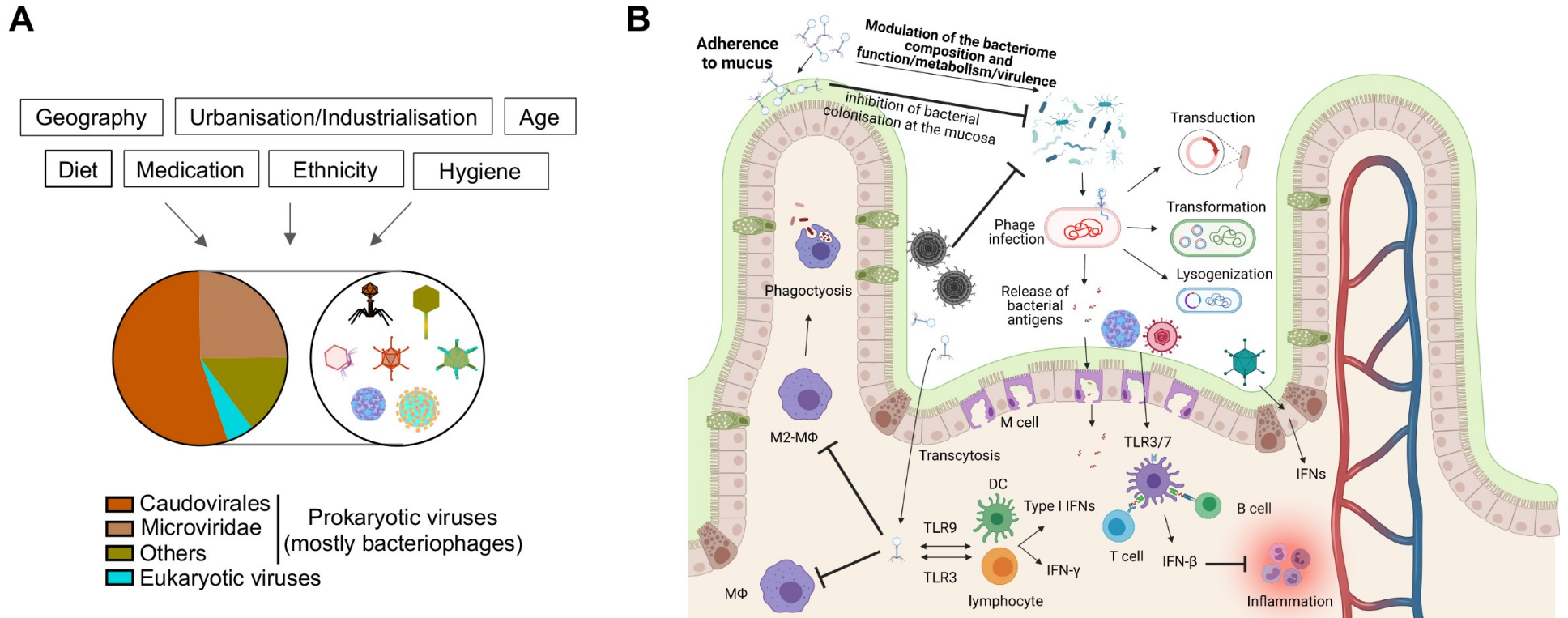
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Figure 1. Composition and function of the gut virome. A. The composition of human gut virome and factors influencing its composition. B.
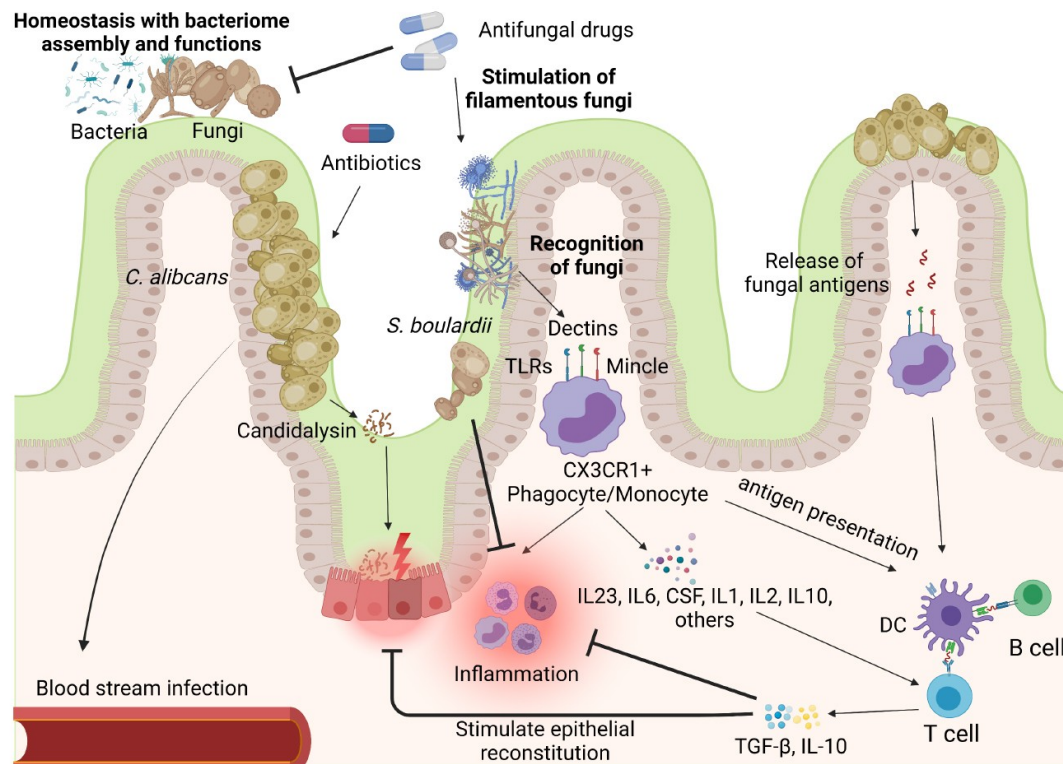Functions of the gut virome on the host.

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Figure 2. Composition and function of the gut mycobiome. A. The composition of human gut mycobiome and factors influencing its composition. B. Functions of the gut mycobiome on the host.

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Table 1. Summary of human studies investigating FMT and gut virome/mycobiome

Indication	Number of study subjects	Type	Methodology	Treatment outcome in association with gut virome/mycobiome alterations	Reference
CDI	N=44	Randomized Controlled Trial	Faecal virome and bacteriome were profiled in association with treatment outcome	*Caudovirales* derived from the donors with increased richness displayed responsiveness	7
	N=55	Randomized Controlled Trial	Faecal mycobiome and bacteriome were profiled in association with treatment outcome	High abundances of *Candida albicans* in donor or recipients led to failure of FMT; FMT decreased *Candida albicans* abundance	8
	N=9	Pilot-study	Analysis in the metagenome, prophage- and CRISPR-based bacteria-phage association and gene functions of the bacteriome and virome after FMT treatment	Successful FMT resulted in functional restoration in the bacteriome and virome resembling donors' profiles. A negative correlation between *Microviridae* and *Proteobacteria* was found before and after FMT	35
	N=1	Case-study	Longitudinal investigations up to 42 months on patient's bacteriome and virome after a successful FMT	Bacteriome and virome in the patient resembled the donor's profile long-term; low phage abundance is associated with a 'healthy' virome profile	47
	N=14	Pilot-study	Investigations up to 12 months on the viral transfer from 3 donors to 14 patients	A successful FMT was associated with durable virome alterations up to 12 months in recipients	46
IBD	N=39	Randomized Controlled Trial	Faecal mycobiome was profiled in association with treatment outcome	FMT responders showed lower faecal *Candida albicans* abundances and anti-*Candida* antibody levels after FMT	9
	N=9	Pilot-study	Analysis in the UC faecal virome and its association with induction of clinical and endoscopic remission	FMT responders showed lower baseline eukaryotic virome richness	44
GvHD	N=1	Case-study	Faecal virome, mycobiome and bacteriome were profiled longitudinally in one patient treated with 4-dose FMT	Decreased faecal fungal diversity after serial FMTs, while virome maintained stable after FMT; relative abundance of Torque teno viruses was decreased, whereas *Caudovirales* bacteriophages was increased in faeces after FMT	12