1 **The human gut virome: form and function**

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14 Running title: Phage form and function

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18 Key words: bacteriophage; human gut virome; human gut phageome

Abstract

Advances in next generation sequencing technologies and the application of metagenomic approaches have fuelled an exponential increase in our understanding of the human gut microbiome. These approaches are now also illuminating features of the diverse and abundant collection of viruses (termed the virome) subsisting with the microbial ecosystems residing within the human holobiont. Here we focus on the current and emerging knowledge of the human gut virome, with a particular focus on viruses infecting bacteria (bacteriophage or phage), which are a dominant component of this viral community. We summarise current insights regarding the form and function of this 'human gut phageome' and highlight promising avenues for future research. In doing so we discuss the potential for phage to drive ecological functioning and evolutionary change within this important microbial ecosystem, their contribution to modulation of host-microbiome interactions and stability of the community as a whole, as well as the potential role of the phageome in human health and disease. We also consider the emerging concepts of a 'core healthy gut phageome' and the putative existence of 'viral enterotypes' and 'viral dysbiosis'.

Introduction

In the last decade we have seen an exponential increase in our understanding of the human microbiome, which may be defined as the collection of microbes associated with the human body and their genetic content. The acceleration in our understanding of this ecosystem has been largely fuelled by advances in next generation sequencing technologies, and the application of metagenomic approaches. These new tools allow detailed, culture-independent, interrogation of the human-microbial landscape at an unprecedented scale, enabling the biological significance and diversity of these human associated microbial communities to be uncovered (1–8).

One of the most well-characterised and densely populated areas of the human microbiome is the adult human gastrointestinal tract. Approximately 10^{13} bacterial cells (9) and an average of ~160 distinct species may reside in the adult human alimentary tract (predominantly in the colon), with over 1000 different bacterial species in total associated with the human gut microbiome (5). The gut microbial community is now accepted to be intimately involved in our health and well-being, providing a range of beneficial functions such as extraction of additional energy from our diet, shaping the development of our immune systems, providing protection from invading pathogens, and has emerging roles in modulating mood, behaviour, neurocognitive development, and even the ageing process (1–4,8,10–15). Imbalances in the make-up of the gut microbiome - also termed dysbiosis – is now increasingly linked with a wide spectrum of diseases and disorders (both gut associated and those relating to extra-intestinal organ systems). These range from Inflammatory bowel diseases and cancer, to metabolic disorders, obesity, and even autism and Alzheimers (16,17). Emphasis is

60   now being placed on delineating whether dysbiosis of the microbiome is a cause or

61   consequence of some of these diseases, and how manipulation of the gut microbiome

62   may aid prophylaxis, diagnosis, or treatment (18,19).

63

64   However, as with microbial ecosystems extant in other habitats, the gut microbiome is

65   itself host to another less well studied and explored community of non-cellular

66   microbes. Metagenomic sequencing efforts are also now revealing the range of

67   viruses associated with the human gut microbiome, termed the human gut virome

68   (20,21). This viral community encompasses an abundant and diverse collection of

69   viruses that infect every domain of life (Eukaryota, Archaea, and Bacteria), but

70   perhaps not surprisingly, is dominated by viruses that infect and replicate within

71   bacterial cells (bacteriophage or phage) (20,21). Because of this dominance of phage,

72   the term "phageome" is often used to refer specifically to the bacteriophage fraction of

73   the gut virome.

74

75   Although the study of the gut virome or phageome is far less advanced than that of

76   the underlying microbiome, a range of important roles and functions have already been

77   attributed to phage in non-host associated bacterial ecosystems (e.g. marine or

78   freshwater environments), which are likely to also apply in some shape or form to the

79   gut virome. These include the transfer of genes between different bacterial strains or

80   species, modulation of community structure and corresponding functional outputs, and

81   provision of accessory functions that directly benefit bacterial hosts (22–24). In the

82   context of host-associated microbial ecosystems, the capacity of phage to endow

83   bacterial hosts with new abilities is of additional significance when traits that may

84   directly impact host health are considered, including toxin synthesis, production of

85   virulence factors and antibiotic resistance genes (20,21,25–32). More generally, the

86   ability to infect and kill their bacterial hosts also give phage the potential to modulate

87   bacterial community structure and destabilise the gut microbiome, which may in turn

88   diminish or obviate benefits provided by the gut microbiome, or lead to deleterious

89   host-microbe interactions (23,33,34). In contrast, recent research is also revealing how

90   phage within the gut virome may play important fundamental functions in microbiome

91   maintenance and recovery from antibiotic perturbation, and the potential for these

92   viruses to enter direct symbiotic relationships with the higher human host (31,35,36).

93

94   As the role of the virome in the development and functioning of the gut microbial

95   community is starting to be uncovered, evidence is accumulating that this viral

96   community also reflects the co-evolution of host and microbe within the gut, driving

97   diversity and functionality, and that specific phage may be unique to or enriched within

98   the gut ecosystem (23,34,37–40). Recognition of these attributes and the potential of

99   phage to drive ecological functioning and evolutionary change (23,34,37,39,40), has

100 understandably ignited interest in investigating the role of these prokaryotic viruses

101 within the human gut virome and as part of the human gut microbiome as a whole. It

102 is also fitting that the concept of dysbiosis, and the impacts of such perturbations on

103 human health, have begun to be considered from the perspective of the virome or

104 phageome, and there is a growing consensus that this concept should also be

105 extended to the phage component of the gut ecosystem (see reviews by (41–43). Here

106 we review current knowledge of the human gut virome (with a particular focus on the

107 phageome), and summarise new insights into its form and function.

108

109 **Virome Structure and ecological dynamics**

110 In recent years, studies of the human gut virome have mainly focused on the analysis

111 of virus like particles (VLPs) purified from faecal samples, and the application of high

112 throughput metagenomic approaches to characterise these (20,21,44,45). These

113 studies have provided much insight into the diversity and structure of the gut virome,

114 which is likely to reflect the underlying diversity of the bacterial microbiome (5).

115

116 Our current knowledge indicates the bacteriophage component of the gut virome (or

117 the phageome) to be dominated by double stranded DNA phages of the order

118 Caudovirales (Podoviridae, Siphoviridae, and Myoviridae) as well as the single

119 stranded DNA containing members of the family *Microviridae* (20,21). These key

120 virotypes mostly infect bacteria belonging to the most prevalent phyla within the gut,

121 comprising members of the Firmicutes, Bacteroidetes, Proteobacteria and

122 Actinobacteria (42). RNA viruses have also been identified, but these appear to

123 represent only a minor fraction of the viral community based on available studies, and

124 are thought to be mainly allocthonous plant infecting viruses ingested with food, rather

125 than perhaps true constituents of the gut virome (27). The adult gut virome may be

126 dominated by just one or a few different virotypes (20,21) and is characterised by a

127 high degree of stability in terms of its structure over time, with temporal tracking of gut

128 virotypes revealing the retention of between 80 to 95% of virotypes over a period of

129 one to 2.5 years (20,21).

130 In terms of diversity, available estimates suggest a healthy human gut is populated by

131 between 35-2800 actively replicating viruses (46), but that phage genome diversity is

132 lower in the gut compared to environments such as the ocean or hot springs and even

133 within other host associated sites, such the lung and oral cavity (47).

134

135   A large proportion of sequences identified in metagenomic surveys, however, are

136   without close homologues within public databases, reflecting the largely

137   uncharacterised nature of the phage gene-space in most microbial habitats, including

138   the mammalian gut. In terms of the gut phageome specifically, the novelty inherent in

139   this viral community was clearly highlighted in the landmark study published by Reyes

140   and co-workers, where approximately 80% of reads lacked notable homology to

141   known viruses in public repositories (20).

142

143   In contrast to other environments, in which phage are known to outnumber their

144   bacterial hosts by an order of magnitude (22), phage are thought to exist at more

145   equitable ratios within the human gut (20), with an estimated 10^9-10^{12} VLPs (48,49) in

146   comparison to an estimated 10^{11} bacteria per gram of faecal material (9,50). However,

147   phage can accumulate to higher densities at mucosal surfaces, significantly

148   outnumbering their hosts in these niches (approximately 20:1 in the murine intestine)

149   (35). Recent work is also revealing that, as with characterisation of the microbiome in

150   general, extraction protocols may influence the estimation of VLP numbers as well as

151   delineation of community structure derived from subsequent high throughput

152   sequencing efforts (48,49). This is also highlighted by studies that have shown

153   significant fractions of the gut phageome may be accessed *via* the analyses of

154   conventional metagenomic datasets, which are based on the extraction and

155   sequencing of bacterial DNA (21,32,51,52). Studies of the gut phageome through

156   these alternative approaches to VLP analysis, have also suggested that standard

157   metagenomes may provide access to particular groups of phage not well represented

158   by VLP-based libraries, which will presumably be dominated by actively replicating

159   phage (32,41).

160

161   Metagenomic analyses have also provided insight into the lifestyles of human gut

162   phage, and provided evidence that the human gut phageome is largely composed of

163   temperate phage, as indicated by the frequency of integrase genes in human gut viral

164   metagenomes (20,21). Temperate phage undergo a lysogenic cycle of reproduction,

165   where phage genetic material is integrated into the genome of bacterial hosts cells to

166   form a dormant prophage, or persists as a dormant episomal element, and replicated

167   with the host genome during bacterial reproduction. The potential dominance of

168   temperate phage in the gut has led to hypotheses that the "piggyback-the-winner"

169   model of phage ecological dynamics pervades in the human gut virome (53,54), in

170   which lysogenic replication is predicted to dominate under conditions of high nutrient

171   availability and bacterial growth. This is in stark contrast to non-host associated

172   environments, in which virulent phage and lytic reproduction appear to dominate,

173   leading to 'kill the winner' (or Lotka-Volterra) phage-host dynamics, that manifest as

174   lagged boom-bust cycles of phage-host abundance (55,56).

175

176   When considered from an evolutionary perspective, the proposed dominance of

177   temperate phage in the gut community is congruent with the reported stability of the

178   gut microbiome in adults (20,21,57,58), and the top-down selective pressure for a

179   functionally stable gut microbiome that is hypothesised to be exerted by the higher

180   mammalian host (4). However, a dominance of temperate, lysogenic phage is also in

181   line with studies that have shown access to novel fractions of the phageome through

182   interrogation of metagenomic datasets derived from bacterial DNA extracts (32,51,52).

183

184   Other models of phage ecological dynamics may also be of relevance to the gut

185   virome, which include "fluctuating-selection-dynamics" (FSD) and "arms-race-

186   dynamics" (ARD) (39,59–61). In the FSD model bacteria and phage populations

187   continually fluctuate though lagged cycles of expansion and contraction, but without

188   total elimination of phage hosts, and instead bacterial diversity is maintained and

189   stable phage communities are established, which is also congruent with features of

190   the gut virome (39,59–61). In contrast, ARD are evident in the co-evolution of lytic

191   phage with host bacteria, in which development of host defence systems to avoid

192   predation by phage are continually countered by reciprocal developments of new

193   infection strategies in phage, leading to what has been termed an evolutionary arms

194   race (39,59). Given the apparent dominance of temperate phage in the human gut

195   virome, ARD seems less likely to be a major ecological dynamic in the human gut

196   habitat, but may be relevant to a limited number of specific host-phage systems, or

197   during situations where bacterial diversity is reduced and underlying ecology of the

198   microbiome altered with respect to the stable adult community. This may include

199   situations such as dysbiosis during disease, and conceivably the infant or elderly gut

200   communities. Readers are directed to excellent reviews (39,59) for more in-depth

201   analysis of phage-bacterial dynamics and effects on the diversity and structure of this

202   ecosystem.

203

204   **Inter-personal variation and potential for 'viral enterotypes'**

205   As with the microbiome, a considerable inter-individual variability in phage diversity is

206   evident in human gut viromes studied to date (20,21,32). This variability is exemplified

207   by the analysis conducted by Reyes et al, who evaluted the representation of highly

208   abundant phage genomes (partial and complete) assembled from metagenomic reads

209   (88 in total), revealing that only 8 were found in more than one individual (20). This

210   high person-to-person variability most likely stems from the ability of phage to undergo

211   rapid evolution to form new virotypes (45), and the associated inter-personal variability

212   of host bacterial species within the gut microbiome (5). In contrast, studies focusing

213   on the microbiome in early infancy have indicted the virome to be low in diversity but

214   highly dynamic during the very early developmental stages (26). Using

215   epifluorescence microscopy, viral particles were found to be absent in the first stool

216   samples from infants, but rapidly appear in the gut and reached levels of up to

217   10^8 particles per gram of faeces by the end of the first week of life (26). A shift in

218   microbiome structure and reduction in species diversity is also now well documented

219   in elderly individuals, but there is currently a paucity of information regarding the gut

220   virome structure in old age, and whether constituent phage may be involved in

221   changes to the gut community seen with ageing.

222

223   Despite the inter-individual differences in adult gut microbiomes and phageomes, the

224   structure of the gut phageome can converge due to diet (21) and individuals (related

225   or unrelated) who live in the same household will share a certain proportion of their

226   gut virome, raising the potential for viral transmission between individuals in close

227   contact (62). More recently, however, a conservation of virotypes in multiple

228   individuals of diverse geographical origin (32,51,52,63) has been detected leading to

229   the hypothesis of a core phageome in healthy individuals (42,63). Despite the reported

230   high level of individuality between human gut viromes (20,21), a set of 23 'core'

231   phages have been identified within more than half of (geographically dispersed)

232   individuals surveyed (n= 62) (63).

233

234 Such shared patterns of phage distribution have opened up the possibility of the

235 existence of viral "enterotypes" (32), akin to observations from the bacterial component

236 of the human gut microbiome, in which multiple individuals could be stratified

237 according to their gut microbial composition into several "enterotypes" (7). As further

238 studies and data accumulate, the nature of, or indeed the existence of these putative

239 enterotypes will be resolved (32,64). Nevertheless, consideration of the human gut

240 phageome should be an integral part of investigations that focus on defining structural

241 or functional aspects of the gut microbiome, and their relevance to human health

242 (32,64).

243

244 **Functional capacity within the phageome and potential for modulation of host-**

245 **microbiome interactions**

246 The idea that phage can also play a role in the health status of individuals by

247 influencing microbiome structure or the phenotypes of host bacterial species, has also

248 started to emerge (41,42,65,47,66). Significant shifts in community structure, including

249 the balance of lytic versus lysogenic phage (see (42), for insightful discussion of this

250 topic), have been observed in conditions such as inflammatory bowel diseases,

251 autoimmunity, leukaemia and diabetes (67–71). How phage may contribute to

252 modifications of underlying bacterial communities, and shifts from equilibrium with the

253 human host to sub-optimal, unhealthy, or pathogenic interactions, is a subject of

254 intense investigation and is comprehensively reviewed by De Paepe and colleagues

255 (47).

256

257 In one scenario, bacterial dysbiosis may result from environmental stressors triggering

258 prophage induction more often in mutualistic bacteria than opportunistic pathogens

259   shifting the ratio of these bacterial types, termed the 'community shuffling hypothesis'

260   (66). Phage within the gut also have the potential to modulate the immune response,

261   either indirectly by modifying bacterial antigens or directly if phage particles are

262   phagocytosed or infiltrate the intracellular environment (47,71,72). Additionally, a

263   potentially unique role in gut health has also been highlighted for phage by recent work

264   in mice revealing that the bacterial and virome components of the murine gut exhibited

265   distinct responses to dietary intervention (73), results that the authors note are

266   reminiscent of responses noted in Crohn's disease patients (69).

267

268   There is also considerable potential for phage to augment the functional repertoire of

269   species comprising the microbiome, and confer particular advantages and new

270   capabilities to host species or strains. This in turn could influence the interaction of

271   these species with the human host, or their competitive fitness within the gut

272   microbiome. Analysis of data generated from human-gut derived VLP libraries, as well

273   as phage-orientated dissection of whole community gut metagenomes, has identified

274   a broad functional potential ranging from bacteriocins, lysins, holins, restriction

275   modification systems, virulence factors, genes associated with energy transfer and

276   key biosynthetic pathways in bacterial hosts, as well as antibiotic resistance genes

277   (20,21,25,26,31,32,74). A functional repertoire that places phage alongside other

278   mobile genetic elements such as plasmids, with regard to the potential to introduce

279   new traits to their bacterial hosts and develop new functional capacity. The ability of

280   temperate phage to form long-term genetic symbioses with their host bacteria, inserted

281   as prophage within host chromosomes, is likely to be an important source of genetic

282   variation and diversity within the human gut, driving genetic exchange and altering

283   phenotypes *via* lysogenic conversion. The apparent dominance of the temperate

284 lifestyle within the gut therefore has profound implications for community development

285 and evolution (as outlined in Table 1), as well as functional capacity of gut microbes

286 (23,75,76).

287

288 There are numerous examples from studies of pathogenic gut bacteria, of phage

289 facilitating the transfer of genetic material and/or encoding functions that may be

290 beneficial for their host bacteria. This includes the wide range of phage-encoded

291 virulence factors and toxins encoded by prophage (77) that lead to major infectious

292 diseases. A particularly pertinent example to the gut ecosystem is the emergence of

293 shiga toxin producing enteropathogenic *E. coli* strains that can cause severe disease,

294 in which toxin production has been acquired through infection with lysogenic phage

295 (78).

296

297 A number of studies are now also revealing a diverse and significant phage-encoded

298 antibiotic resistance gene pool within the human gut (20,21,79), which has been

299 shown to be viable, mobile (31) and widely distributed within multiple individuals of

300 diverse geographical origin (32). Moreover the importance of phage and the

301 mechanisms used for acquiring and distributing antibiotic resistance genes has also

302 been highlighted (80,81). Nevertheless, the true extent of the phage-encoded

303 antibiotic resistance pool needs to be charted in a systematic and stringent manner.

304

305 In this context, Enault and colleagues (82) have suggested that the use of non-

306 conservative thresholds within many bioinformatic-based surveys may have led to vast

307 overestimates of phage-encoded antibiotic resistance genes in virome studies. The

308 authors argue that the main route of transfer will be via generalised transduction, which

309   relies on errors in the packaging of non-phage DNA, intrinsically limiting the role of

310   phage in dissemination of these genes to movement between species or strains

311   infected by the transducing phage (82). How these observations can be reconciled

312   with the reported expansion of the phage resistome following antibiotic perturbation

313   ((31); see also *Community-level functions of the gut phageome*), has yet to be

314   determined. However, it is clear that further investigations of the mechanisms of

315   phage-encoded gene transfer of clinically important traits such as antibiotic resistance

316   are required to provide a more cohesive view of gene transfer and phage-bacteria

317   interactions within the gut. Such studies will be inevitably aided by the isolation and

318   genomic characterisation of phage originating from the human gut and in particular

319   those infecting key members of this microbiome.

320

321   Aside from the acquisition of accessory functions, such as virulence or antibiotic

322   resistance genes, phage also have the potential to influence strain-strain competition

323   within the gut ecosystem by facilitating the elimination of strains that would otherwise

324   compete with bacterial hosts for resources. This derives from situations where

325   prophage confer immunity to host strains from further infections by the same or closely

326   related phage (30; Table 1). This contribution of phage to strain-strain competition

327   could also play a role in facilitating maintenance of particular strains within the gut

328   microbiome, or potentially in the ability of less desirable strains to displace beneficial

329   members of the gut community. An elegant example of phage-mediated inter-strain

330   warfare has recently been described in *Enterococcus faecalis* V583 (30). Prophage

331   carried by this human gut colonising strain have been found to be crucial in allowing it

332   to persist in the intestine, and avoid competitive exclusion by other strains. In this case,

333   the prophage carried by V583 produces a constitutive low-level shedding of viral

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334 particles from the host cell (also referred to as a chronic replication cycle). The phage

335 particles release by V583 can infect and kill competing strains, but V583 host cells are

336 rendered immune by virtue of their prophage lysogen (30). Alternatively, carriage of

337 cryptic phages – a prophage that has lost the ability to enter the lytic cycle or produce

338 phage particles – can be beneficial for surviving adverse conditions. The carriage of

339 cryptic phage by *E.coli* enables the host bacterium to withstand multiple stressors such

340 as oxidative, pH and antibiotic stressors and influences growth and biofilm formation

341 (83; Table 1).

342

343 **Community-level functions of the gut phageome**

344 There is also considerable interest in the potential for the human gut phageome to play

345 important and fundamental roles in the maintenance of a stable gut microbiome, and

346 the scope for these viruses to provide direct benefits to the human host, outside of

347 those that may be gained indirectly through effects on the bacterial gut community (as

348 summarised in Table 1; see (84) for discussion of the beneficial impacts of viruses).

349

350 The potential for phage to contribute to adaptability and recovery of the gut microbiome

351 has been recently highlighted by Modi and co-workers, who detail the functional

352 resilience of the community following antibiotic exposure (31). Gut-derived phage

353 metagenomes from mice subjected to stress in the form of antibiotic treatment, were

354 enriched in genes encoding functions involved in host metabolism, such as

355 metabolism of cofactors and vitamins, as well as carbohydrate degrading enzymes

356 and antibiotic resistance determinants. The authors suggested that this phage-

357 encoded accessory gene pool could act as a community-based mechanism to

358 preserve the functional robustness of the gut microbiome during perturbation, acting

359   as a buffer of functions that are essential for the host-microbe relationship and that

360   may become depleted during insults such as antibiotic treatment. The perturbation

361   also led to a reported expansion of interactions between phage and bacteria that would

362   likely increase the possibility of gene exchange, and further affirm the well-known role

363   of phage in horizontal gene transfer. See Sun & Relman (85), for further analysis of

364   this topic.

365

366   Moving beyond the direct phage-bacteria co-evolutionary relationship, the potential for

367   phage to directly enter into a symbiotic relationship with higher metazoan hosts has

368   also been documented in some host-microbe relationships, and theorised to occur

369   within the human gut (35,36; Table 1). In these phage-metazoan relationships,

370   protecting or enhancing the fitness of the metazoan benefits the phage by maintaining

371   the habitat, and therefore population, of host bacterial species in which it replicates. A

372   good example of this co-evolutionary triangle has been identified through studies of

373   the aphid symbiont *Hamiltonia defensa* (36), which confers protection to aphid hosts

374   from attack by parasitoid wasps. Detailed analyses of this symbiosis has revealed the

375   toxins responsible for the *H. defensa* protective effect are encoded by lysogenic phage

376   that infect this species, and strains of *H. defensa* lacking this phage lysogen provide

377   no protective effect (36).

378

379   In the context of the human gut microbiome and associated virome, hypotheses for

380   how this collection of phage may conceivably provide direct benefits to the human host

381   are also emerging. The apparent enrichment of phage in the mucus layer covering

382   intestinal epithelial cells in the mammalian gut, has led Barr and co-workers (35) to

383   propose that phage accumulating in this region may provide important benefits for the

384 host by controlling the bacterial population in the mucus layer, and potentially providing

385 protection from pathogens. Within this theory, phage embedded within the intestinal

386 mucus layer are essentially hypothesised to provide a form of "non-host-derived

387 immunity", and enter into a direct symbiosis with the higher mammalian host (35). If

388 true, this function of the gut virome opens further routes though which gut-associated

389 phage may not only influence aspects of human well-being, but also potentially

390 aspects of development, and would suggest that understanding the capacity or

391 potential for "viral dysbiosis" should be given the same emphasis as perturbation of

392 the bacterial component of this community. An exhaustive review of the concepts and

393 potential consequences of host-microbiome-virome co-evolution is beyond the scope

394 of this review but we refer the reader to excellent reviews by (39,59).

395

396 **Applications**

397

398 Since their initial discovery in the early twentieth century independently by Frederick

399 Twort in 1915 (86) and Felix d'Herelle in 1917 (87), phage have been viewed in terms

400 of human application, in the first instance for their antimicrobial properties. There is a

401 long history of lytic phage and their encoded products being used to kill pathogenic

402 bacterial species, an approach currently entering a renaissance in this critical era of

403 antibiotic resistance. See (88–91) for excellent discussions on the topic. In the context

404 of biotechnological and biomedical applications, phage also have a long history as

405 work horses of molecular biology research, as well as delivery vehicles for vaccines

406 and gene therapy (92). As our ability to chart and characterise the human gut

407 phageome (and virome as a whole) continues, however, new biomedical and

408 biotechnological avenues of application are being pursued.

409

Recognition that dysbiosis may also incorporate the phage component of the human gut ecosystem also opens up novel options for using phage as prognostic or predictive markers of disease (41,43). The notion that individuals could be stratified according to their virome structure (32) creates potential to develop diagnostics, prognostics and treatment approaches (64), expanding the microbiome for personalised medicine applications (7,93). In tandem, the idea that phage have the potential to contribute to recovery and resilience of a gut community following perturbation (23,31) forms the foundation of using virome components for targeted interventions. In this context there has been much interested in the genetic engineering of phage for microbiome restructuring and selective elimination of bacterial strains (94–96).

420

The specificity of phage for the human gut (38,97) can also be leveraged for the development of microbial source tracking tools (MST), which permit detection of faecal pollution in surface and ground waters. Faecal contamination poses a significant risk to human health and therefore there has been much interest in developing rapid and sensitive culture-independent methods that can detect human faecal indicator phage within environmental samples. Phage persist longer in the environment than host bacteria and often at higher numbers making them a potentially more sensitive source tracking tool (98–101). In tandem with improvements in next generation sequencing technologies, there is also potential to develop metagenomic approaches to MST, e.g. (102,103).The combination of MST with the high resolution provided by metagenomics.-based analysis of whole microbial communities potentially enabling identification of habitat associated genetic patterns – or an ecogenomic signature - that could be used to differentiate ecosystems.

434

Conclusions

Knowledge around the human gut virome is now accumulating at an increasingly rapid pace. In particular next generation sequencing technologies have given us the ability to access the human gut phageome (and virome in general), to gain fundamental insight into the form and function of this viral assemblage. Studies to date are revealing a diverse and abundant viral ecosystem that is being increasingly recognised as an important facet of the gut microbiome. As progress continues in our ability to characterise the human gut virome so does our ability to harness its power for biomedical and biotechnological application. Further understanding of the host-phage dynamic is crucial in moving the field forward, with more emphasis on temporal and multi-level studies that take a more inclusive view of the human gut virome, alongside the other components of the human gut microbiome. Integral to these efforts, and the more meaningful interpretation of data derived from metagenomic approaches to study the gut virome, will be continued efforts to pursue more traditional approaches to isolate, propagate, and characterise phage comprising the human gut phageome.

Summary Points

- Advances in next generation sequencing technologies are allowing us to illuminate the human gut virome, the diverse and abundant collection of viruses associated with the human gut microbiome.

- We focus here on the form and function of the viruses infecting bacteria (bacteriophage or phage), which are a dominant component of the human gut virome.

- Key points of discussion include the potential role of the 'phageome' in driving ecological functioning and evolutionary change with the human gut microbiome and how they could contribute to modulation of host-microbiome interactions and stability of the community as a whole, as well as their potential role in health and disease.

- Emerging concepts of a 'core healthy gut phageome', the putative existence of 'viral enterotypes' and 'viral dysbiosis' are considered.

468   **Box 1**

Glossary	
Bacteriophage	Viruses which infect and complete their life cycle within bacterial cells
Human gut virome	The collection of viruses associated with the human gut microbiome
Phageome	The genetic composition of the bacteriophage fraction of a microbial community
Prophage	Term to describe a phage genome which is integrated into a bacterial chromosome. Can also exist externally to the bacterial chromosome as an episomal element. The prophage is a non-infective precursor phage.
Temperate	When phage have the ability to enter a lysogenic cycle of replication.
Lysogeny	Ability of phage to integrate into host bacterium DNA to become a prophage. The prophage is copied each time the bacterial cell divides.
Lysogenic conversion	Expression of prophage genes within the host genome affecting the phenotype of the host cell.
Composite phage	A phage derived from two distinct chromosomally encoded prophage elements

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745 **Conflict of Interest Statement**

746 The authors declare that the research was conducted in the absence of any

747 commercial or financial relationships that could be construed as a potential conflict of

748 interest.

749