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The Viruses of the Gut Microbiota - from Pathogenic to Commensal

(Title of Thesis)

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ABSTRACT

Clinical virology has been steadily on the rise with the advent of next-generation sequencing, bringing new knowledge on the gut virome. The gut virome is part of the microbiome, which, along with bacteria, archaea, fungi, and protozoa, are a variety of microorganisms that maintain homeostasis of body and cell functions. Evidence of this diverse and abundant population of viruses in the gastrointestinal (GI) tract has confirmed the existence of commensal viruses, which comprise the gut virome.

There is evidence supporting the association of chronic inflammatory bowel diseases such as Crohn's disease and ulcerative colitis with the disruption of the gut microbiota homeostasis, and the role of viruses particularly bacteriophages on these chronic illnesses and treating antibiotic-resistant diseases. In this literature review, the importance of viruses and their effect on gut health and disease is highlighted. The scientific information is coming but still there is huge need for new data regarding importance of commensal virus on human health.

Keywords: Microbiome, Microbiota, Gut Virome, Bacteriophages, Commensal Viruses.

Microbiome: The microbiome is the community of microorganisms (such as fungi, bacteria and viruses) that exists in a particular environment, a collection of genomes from all these microorganisms including structural and metabolic elements.(1,3)

(In humans, the term is often used to describe all the microorganisms that live in or on a particular part of the body, such as the skin or gastrointestinal tract. These groups of microorganisms are dynamic and change in response to a host of environmental factors, such as exercise, diet, medication and other exposures.)

Microbiota: are a collection of microorganisms found within a defined environment for example the Gut microbiota, respiratory tract microbiota, urinary system microbiota (1,3)

Gut Virome: the total population of viruses, including bacteriophages and eukaryotic viruses, found in the human gastrointestinal tract.(1,3)

Bacteriophage: a particular type of virus that infect bacterial cells including resident microbiota.(1,3)

Commensal Viruses: viruses that benefit from their host but neither harm nor help it. They are a part of the human virome, contribute to the overall health of the host by interacting with the immune system and microbiota.(1)

INTRODUCTION

The advancement of medical science has led to many discoveries that have proven to be beneficial to our understanding of how the human body works. One such discovery was the presence of a microbiome in the gut— a collection of microorganisms in the entire gastrointestinal tract.

Despite their frequent interchangeability, the terms "microbiota" and "microbiome" have some distinctions. The term "microbiota" refers to the live microorganisms, such as gut and oral microbiota, that are present in a certain environment. The term "microbiome" describes the collection of genomes from all the microorganisms found in the environment, which includes the microbial population as well as the metabolites, structural components, and environmental factors. In this sense, the microbiome is more diverse than the microbiota.(28)

Gut microbiota plays a crucial role in numerous processes for example in maintaining homeostasis within the body, such as metabolism and immune development.(1) Almost 2000 years ago, Hippocrates, father of medicine coined the famous phrase " feed a cold, starve a fever" as Hippocrates understood starving the sick person would starve the fever and therefore illness. This phenomenon has garnered much attention over the years with the current times as we now have the scientific tools to study to support this hypothesis and its association with the overall physiological health of an individual.(2)

With the increasing support from research, we now have proof of the human body harbouring commensal resident microbiota not only within the gut but also found on the surfaces of the mouth, skin, and urinary tract. This resident microbiota is regulated by several factors such as host genetics, environment, diet with mode of delivery being the single largest influencer on the development of the host microbiota and consequently the virome.

Studies have also shown that evolutionary pathways have shaped the commensalism of the host and its corresponding microbiota, as they mutually gain benefits from each other. The microbiota can induce physiologic, metabolic, and immunological regulations that the host can benefit from, while the host in turn provides a site for the microbiota to gain nourishment and propagate itself.(2)

To illustrate a few examples, the gut microbiota is known to have a profound effect on the development and function of the host's immune system, as referenced by an experimental study involving mice, which demonstrated decreased number of gut-associated lymphoid tissues, smaller

and fewer mesenteric lymph nodes and Peyer's patches, reduced secretory immunoglobulin A (IgA) production, and abnormal intestinal T cell development in mice without the presence of commensal microbiota.(2)

In mammals, the gut microbiota accounts for about 8% of resting energy expenditure, and changes in the gut microbiota led to increased weight gain over a period of months. A popular inbred strain of laboratory mice, C57BL/6 mice are frequently employed as model organisms to study metabolic diseases such as diabetes and obesity. These mice exhibit aberrant glucose metabolism and microbial composition, which are hallmarks of type 2 diabetes.(10)

It has been demonstrated that faecal virome transplantation (FVT) can ease such symptoms and change the gut microbiota, which delays the onset of obesity in people who consume high-fat diets. It has been alluded that a change in the viral population from virulent to temporal phages or the restoration of lost phages that regulate the microbial communities could be responsible for this change.(10)

Since humans and microbiota co-evolved, the human immune system and bacteria have a complicated symbolic relationship. The child's first exposure to microbiota is thought to be the vertical transmission from the mother's microbiota during delivery. Babies born via caesarean section are colonized with bacteria of the epidermal origin, which may increase their risk of developing allergies and asthma, compared with infants who received initial microbiota from the maternal vaginal flora.(28)

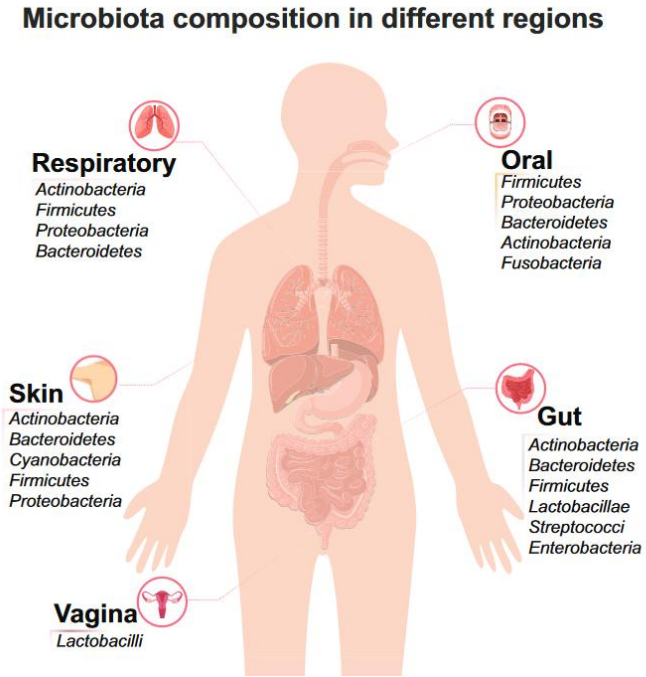


Figure 1 Human microbiota composition in different locations. Predominant bacterial genera in the oral cavity, respiratory tract, skin, gut, and vagina are highlighted. (Hou et al, 2022)

The human gastrointestinal tract is an organ that starts at the mouth and ends at the anus, passing through the eosophagus, stomach, and intestines, and is home to a variety of microorganisms that make up the microbiome. This includes the bacteria, archaea, fungi, protozoa, and viruses (1)

Viruses are categorized according to their size, shape, genomic structure, chemical makeup, and reproduction method. DNA or RNA, either single stranded (ss) or double stranded (ds), linear or circular, can make up a virus's genome. One nucleic acid molecule (monopartite genome) or multiple nucleic acid segments (multipartite genome) may contain the full genome. Different replication techniques are required for the various genome types. (3)

Virome and (meta)viromics are two new "-omes" and "-omics" that viruses have acquired in recent years. All viruses that live in an ecosystem are included in these words, as are their genomes and the study of them. There are numerous methods to categorize these viruses, one of which is by their host. (21)(**Error! Reference source not found.**)

The Baltimore classification divides viruses into seven groups: double-stranded DNA viruses (Group I), single-stranded DNA viruses (Group II), double-stranded RNA viruses (Group III), positive single-stranded RNA viruses (Group IV), negative single-stranded RNA viruses (Group V), and positive single-stranded.(44)(Table 1)

Table 1 Baltimore Classification

Baltimore Classification			
Group	Characteristics	Mode of mRNA Production	Example
I	Double-stranded DNA	mRNA is transcribed directly from the DNA template	Herpes simplex (herpesvirus)
II	Single-stranded DNA	DNA is converted to double-stranded form before RNA is transcribed	parvovirus (parvovirus)
III	Double-stranded RNA	mRNA is transcribed from the RNA genome	Childhood gastroenteritis (rotavirus)
IV	Single stranded RNA (+)	Genome functions as mRNA	Common cold (picornavirus)
V	Single stranded RNA (-)	mRNA is transcribed from the RNA genome	Rabies (rhabdovirus)
VI	Single stranded RNA viruses with reverse transcriptase	Reverse transcriptase makes DNA from the RNA genome; DNA is then incorporated in the host genome; mRNA is transcribed from the incorporated DNA	Human immunodeficiency virus (HIV)
VII	Double stranded DNA viruses with reverse transcriptase	The viral genome is double-stranded DNA, but viral DNA is replicated through an RNA intermediate; the RNA may serve directly as mRNA or as a template to make mRNA	Hepatitis B virus (hepadnavirus)

The International Committee on Taxonomy of Viruses (ICTV), has been the sole body charged with classifying viruses since 1966 and they classify viruses according to the virion size, capsid structure, type of nucleic acid, physical properties, host species, or disease caused.(43)()

Table *2*)

As of right now, the ICTV has classified viruses into seven orders, each of which comprises 103 families. Notable virus families including the retroviruses, papillomaviruses, and poxviruses are among the seventy-seven virus families that have not yet been placed in an order. There have been suggestions for new orders, and as the taxonomy process progresses, more will probably be developed.(43)

Table 2 International Committee on Taxonomy of Viruses classification of Viruses. (Louten et al, 2018)

Order	Notes
Caudovirales	Tailed dsDNA viruses that infect members of the domains Bacteria and Archaea; name comes from Latin *cauda*, meaning "tail."
Herpesvirales	dsDNA viruses of vertebrates and invertebrates; from Greek *herpes*, meaning "creeping" or "spreading" (describing the rashes of these viruses).
Ligamenvirales	dsDNA viruses that infect the domain Archaea; from Latin *ligamen*, meaning "thread" or "string" (describing the linear structure of the viruses). Newest order, created in 2012.
Mononegavirales	"Negative-strand" ssRNA viruses of vertebrates, invertebrates, and plants; name derives from Latin for "one negative," referring to the single negative-strand RNA genome. Was the first order created, in 1990.
Nidovirales	"Positive-strand" ssRNA viruses of vertebrates and invertebrates; from Latin *nidus* meaning "nest" because they encode several proteins nested within one piece of mRNA.
Picornavirales	"Positive-strand" ssRNA viruses of vertebrates, invertebrates, and plants; from pico (small) + RNA + virales (viruses).
Tymovirales	"Positive-strand" ssRNA viruses of plants and invertebrates; Tymo is an acronym standing for Turnip Yellow Mosaic virus, found within this order.

Pertaining to the human microbiota, it was previously thought to be composed of only bacteria, but recent studies have given credence to the theory that a great diversity of viruses also exist commensally within the gastrointestinal tract, namely the virome.

Bacteriophages are specific viruses that infect bacteria which were first identified by Frederick Twort and Felix D'Herelle in 1915 and 1917 respectively and yet we are only discovering their role in health and disease.(24) It is also important to note that the human virome or bacteriophage is present not only in the gastrointestinal tract, but are distributed widely throughout the human body, as characterized by several studies.(3)(Figure 2)

Modern research methods using advanced biotechnology and next-generation sequencing has enabled our understanding of the gut microbiota and virome, revealing that the viral population in the gut may match or exceed bacterial populations. The gut virome includes diverse viral types, with 90% being prokaryotic viruses and 10% eukaryotic viruses, as well as DNA and RNA viruses. The limited literature on gut RNA viruses is due to their instability in cultures and the individual specificity of gut viral composition.(4)

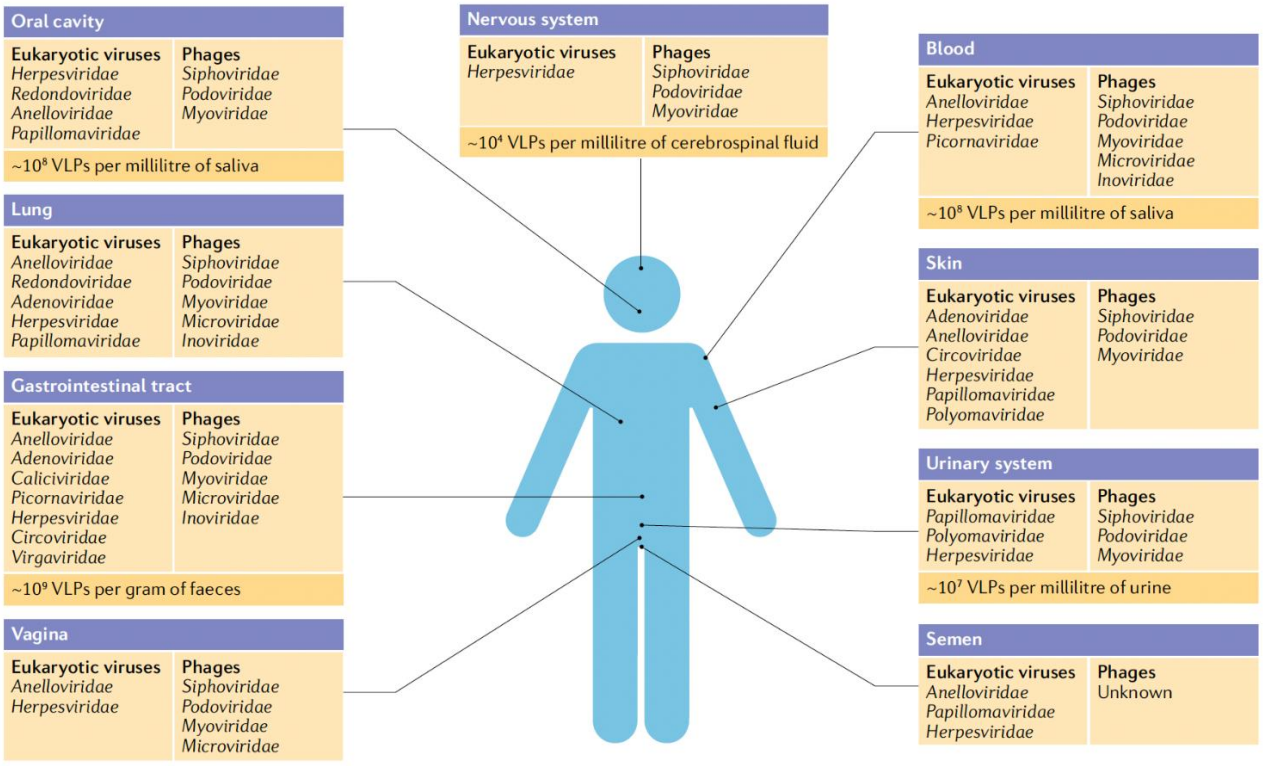


Figure 2. An overview of the viruses discovered in each part of the human body.(Liang & Bushman, 2021)

Viral types are compiled from published virome surveys. As more human populations are surveyed and new viral types are identified, the most prevalent, and well researched, well-known, family is *Caudovirales*, which has lengthy, non-enveloped tails and the subcategories *Siphoviridae*, which have noncontractile and rigid/nonflexible tails; *Podoviridae*, which have short noncontractile tails; and *Myoviridae*, which have contractile, complex baseplate long flexible tails.(10) Viral lineages were occasionally left out of the analysis since they might be contaminants or misattributions. *Mimivirus, Phycodnavirus, Marseillevirus, Flaviviruses, Poxviruses, and Baculovirus* in the vagina are among these exclusions.(2,)

The difficulty in ascertaining viruses and subsequent studies have been a barrier to the gut virome gaining widespread attention. However, modern research has made it possible for scientists to learn

that bacteriophages, being the most abundant members of the gut virome, are likely to have a substantial impact on the human host.(10)

In recent years, the understanding that a well-functioning microbiota, including virome, is necessary for human health has broadened researchers' insights towards the interactions between microbiota, virome, invading pathogens and the rise in antibiotic resistance.(2,10)

This literature review focuses on the characteristics and function of the gut virome, along with its microbiome components. Furthermore, an exploration regarding its potential therapeutic application including faecal transplantation to alleviate distressing symptoms brought on by chronic illnesses for example in irritable bowel disease are explored.

For the literature review, I used standard search strategies involving the querying of online databases including, Global Virome Database, Gut Phage Database, PubMed Centra, Cochrane, using key words such as Bacteriophage, Gut Virome, followed by evaluation of the bibliographies for relevant articles within 1980-2025 time period.

CHAPTER 1: Commensal Viruses

More than two decades ago, an editorial by P. Griffiths postulated that "we should not exclude the possibility that commensal viruses may exist". That was then the forefront of scientific literature on virology.(5,25).

In the present decade, virological studies have gained traction with next-generation sequencing, particularly in characterising viral genomes and in turn, viral aetiologies for numerous conditions and diseases such as Merkel cell polyomavirus, and astrovirus in central nervous system infections. This particular branch of science has also been useful in identifying and characterizing emerging viral strains, most notably the Middle East respiratory system coronavirus (MERS-Cov) and the Ebola virus, to name some examples.(5)

However, as scientists are now discovering which virus is responsible for a particular disease, there is also abundant research detailing the presence of viruses in otherwise normal and healthy subjects. Viral sequences have been shown to appear in asymptomatic control patients, and even in those immunocompromised patients without symptoms of an overt infection. This wealth of evidence points out that there is indeed a plethora of viral populations that exist in the human body as commensal viruses.(5)

Gut Viruses include Eukaryotic and prokaryotic viruses, such as those that infect human cells, microorganisms (such bacteria, fungus, and archaea), and plant viruses that are mostly obtained from the environment and diet. In the pathophysiology of diseases such as inflammatory bowel disease (IBD), bacterial microbiome (bacteriome), Clostridium difficile infection (CDI), obesity, diabetes, SARS-CoV-2 infection, liver diseases, colorectal cancer (CRC), and malnutrition, the gut virome is also crucial.(1)

To provide further examples of these commensal viruses, The *Pegivirus*, a spherical enveloped virus previously named as the hepatitis G virus (HGV) or GB virus C (GBV-C), has been found to be present in 1%-5% of healthy blood donors. Viral sequence analyses discovered that these two isolates were the same virus, and it does not cause hepatitis disease despite sharing some genome organization with the hepatitis C virus.

The *Pegivirus* is found mostly in lymphocytes and not hepatocytes, and several studies found that infection with *Pegivirus* was associated with prolonged survival in people living with HIV. (5)

Follow-up studies showed that concurrent infection of human lymphocytes with the *Pegivirus* reduces the immune activation of T cells, B cells, natural killer cells and monocytes, ultimately leading to reduced progression of HIV infection and HIV-associated mortality. This became a well-known example of how the human virome can be a commensal organism, rather than Koch's monocausal dogma of infectious disease.(5)

Another example, the *Torque Tenovirus*, a non-enveloped, single-stranded, circular human DNA virus, has been identified as a surrogate marker of immune competence in solid organ transplantation, although the exact method by which this happens remains a point of debate.(5)

As such, many studies have proven that viruses can exist within the human microbiome without causing disease, existing in a kind of equilibrium, or homeostasis, within the host’s immune system.

CHAPTER 2: Composition of the Gut Virome

We now know that the human body has immense population of commensal viruses that exist within various body sites, however for the purposes of this literature review will focus on the gut virome in particular the Bacteriophage.(1,3)

The virome is established shortly after ,after birth when the newborn is exposed to a non-sterile environment and viral colonization takes place. Method of birth, either by Spontaneous Vaginal Delivery (SVD) or Caesarean Section (CS), is the single most influential factor major in the

composition and diversity of the neonate microbiota, bacteriophage and virome communities. It has been established that infants born via vaginal delivery exhibit a greater diversity in their bacteriophage composition where *Caudoviricetes*, *Microviridae*, and *Anelloviridae* were the most abundant viruses detected in babies delivered via vaginal delivery compared with caesarean section. (23)

310 CS and 281 SVD babies' gut microbiota samples were used in a recent longitudinal study that showed distinct and noteworthy variations in the microbiomes by mode of birth and variations brought on by prenatal prophylactic antibiotic use.(4,6)

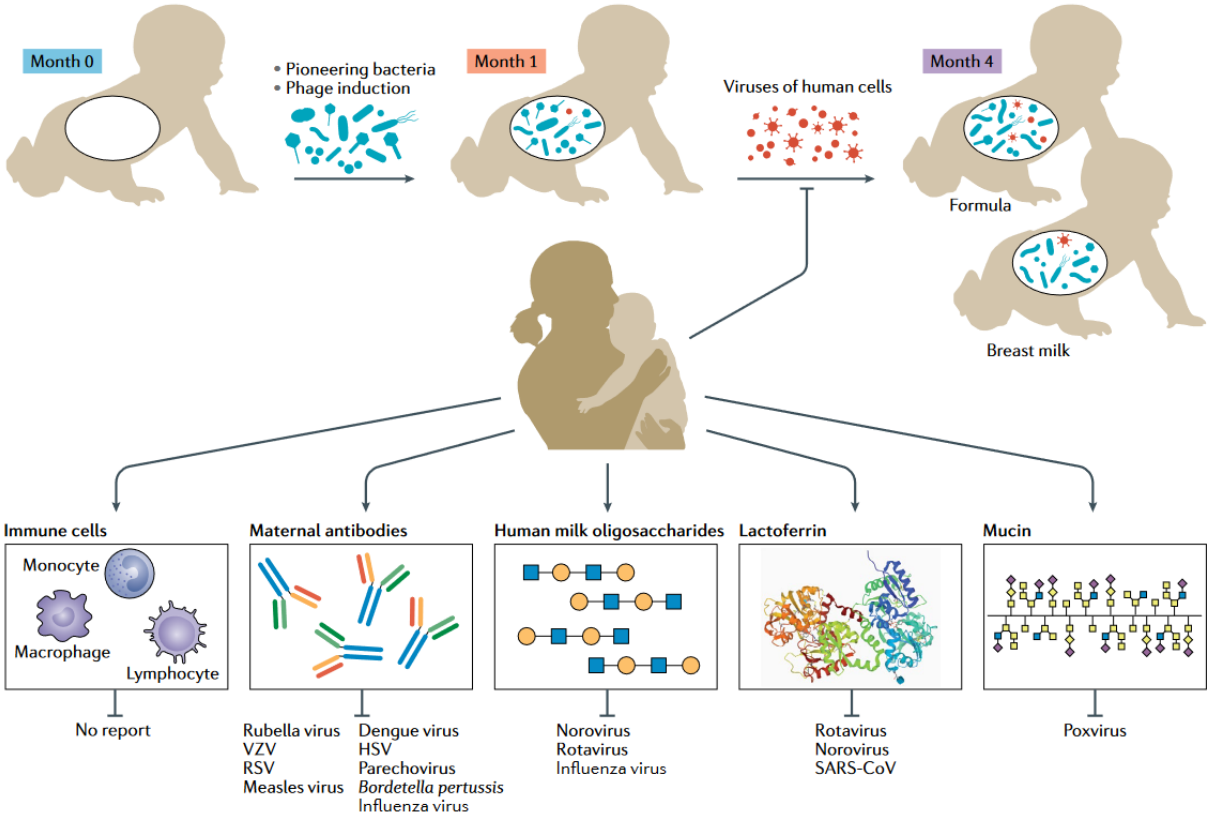


Figure 3 Step wise assembly of the gut virome.(Liang & Bushman, 2021)

Healthy neonates are typically born lacking a gut virome or microbiome. Pioneering bacteria colonize the gut of the neonate, such that infants have a detectable microbiome by month 1 of life. These bacteria commonly harbour integrated prophages, which occasionally induce prophages, providing a first wave of viral particles in the gut. Later, by month 4, more viruses that infect human cells can be detected. Infection with these viruses, some of which can be pathogenic, is inhibited by breastfeeding. Breastfeeding can also alter the types of phages present by altering the proportions of bacteria in the

infant gut, which consequently alters the proportions of their phages. The protective effects of breast milk can be conferred by maternal immune cells or various macromolecules. These include maternal antibodies, human milk oligosaccharides, lactoferrin, mucin and gangliosides. The viral groups targeted by each antiviral factor in human breast milk are shown. HSV, herpes simplex viruses; RSV, respiratory syncytial virus; SARS-CoV; severe acute respiratory syndrome coronavirus; VZV, varicella zoster virus. (3)

It would be imprudent not mentioning the effect of Diet and the impact this has on the microbiome including the virome diversity found within the gastrointestinal tract. An experimental study was done in 2011 by a group of researchers in Pennsylvania to understand the composition and dynamics of the human virome as affected by the individual's diet.(4,5)

According to the Global Virome Database, 97.7% of the human gut virome are bacteriophages, 2.1% are eukaryotic viruses, and 0.1% are archaeal viruses; 88% of these phages have yet to be classified by the International Committee on Taxonomy of Viruses.(6)

Two most crucial for the diversity of the microbiome population which in turn guides the health and development of the neonate was attributed to Mode of Delivery and Diet. Early feeding choice is a well-established role with babies who are breast-fed display a more diverse microbiota population including eukaryotic viruses and bacteriophages than those formula-fed. (3,6)

This diversity is due to the antibodies transferred through breastfeeding and transmission of milk phages to the infant GI tract that help shape the infant GI microbiota. (21)

Growth of Specific bacteria, e.g. *Bifidobacteria, Lactobacillus*, and *Streptococcus* is enhanced with breastmilk as it contains a variety of complex macronutrients composed of lipids, proteins and carbohydrates, immune cells and antibodies, as well as human milk oligosaccharides. (22). It's no surprises that breast milk is sometimes referred to as "liquid gold" with many health institutions globally providing breast-milk banks.(6)

In newly delivered infants, the gut virome is comprised mostly of bacteriophages that infect the first bacteria in the gastrointestinal tract of newborns. This is followed by the proliferation of eukaryotic viruses which is mostly from breast milk. The single stranded DNA viruses of the *Anelloviridae* (particularly *Torque Teno Virus* species) were reported to be the most abundant in the few months after birth. This may be due to the immature host immune system, and its reduction in the subsequent years correlates to a more developed immune system.(6.7)

Up to age three, the predominance of DNA phages from the *Caudiovirales* order, mostly comprising of *Myoviridae, Siphoviridae* and *Podoviridae* family has been documented. This is mostly attributed to early colonization of the gut by Bacteroides, Proteobacteria and Actinobacteria bacteria.,(7,23)

CrAssphages, (cross-assembly phage) which are associated with Bacteroides, showed an overall upward trend with age. They were found to be abundant and persistent in the human gut virome, and functions to maintain a stable population of both resistant and sensitive bacterial hosts stabilizing the virome population with added specificity.(7,23). This pattern highlights the dynamic nature of the gut virome and its potential impact on human health across our lifespan.(4)(Figure *5*)

Cao et al. reported that the gut virome, particularly the bacteriophage population, follows a pattern wherein infants (0-3 years old) and adults (18-65 years old) show a greater viral richness while it decreases during childhood (3-18 years old) and the elderly (65 and above).(1)(*Figure 4*)

Estimates show that there are approximately 35 – 2800 active bacteriophages in 1g of faeces, which reflects the overall diversity of viruses throughout the entire gastrointestinal tract.(6)

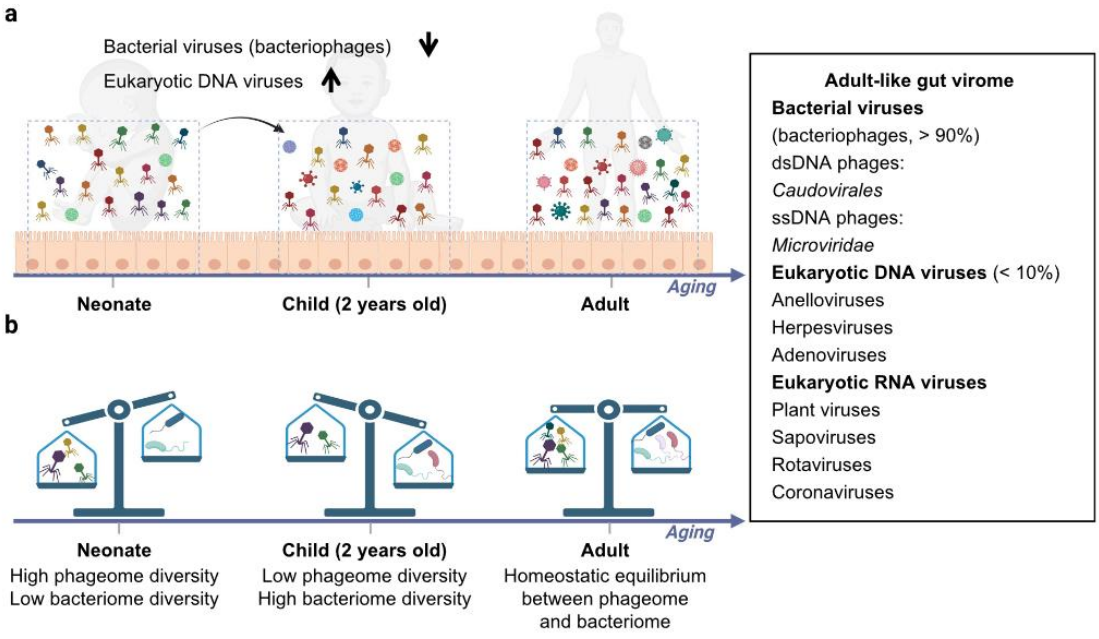


Figure 4 The composition of the human gut virome. (Cao et al,2022)

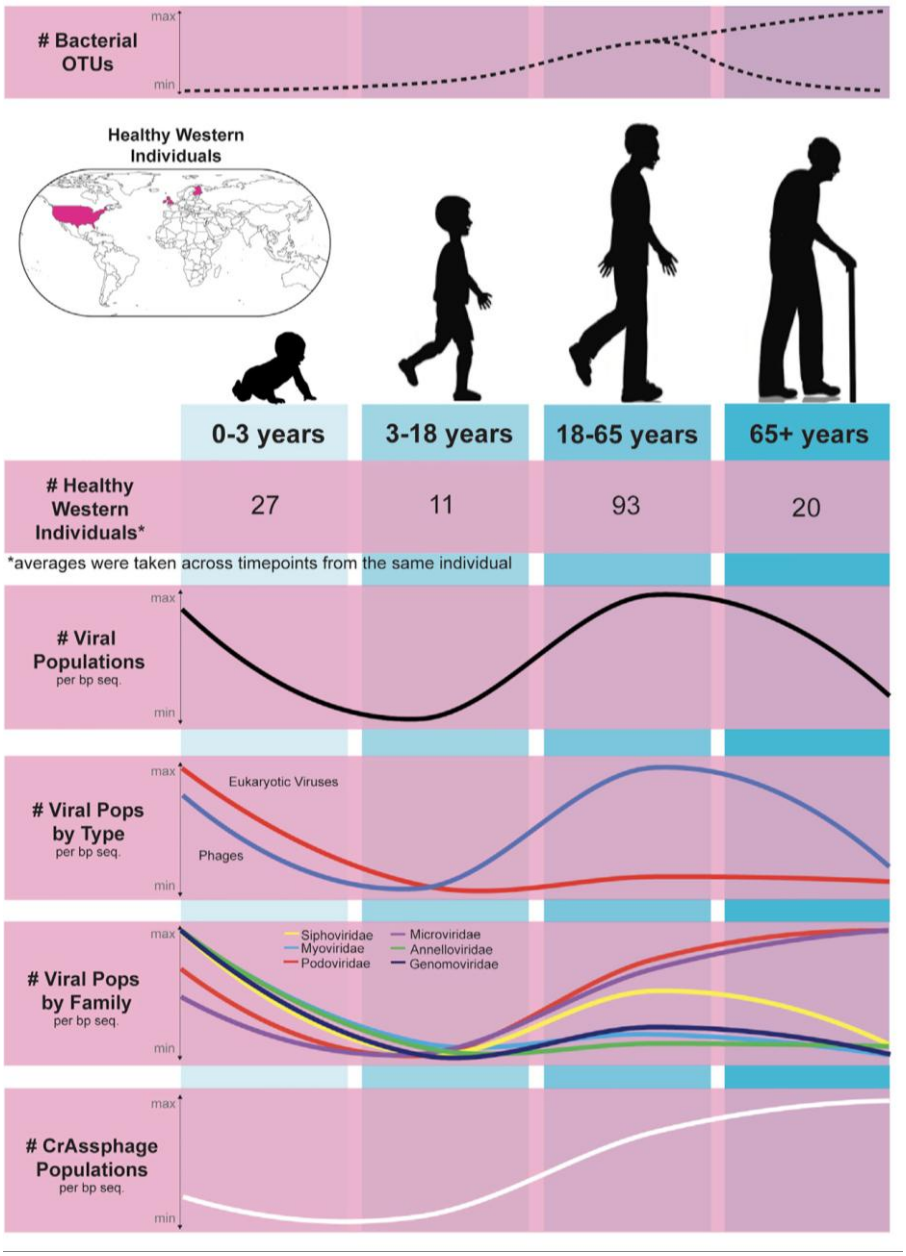


Figure 5. Viral diversity across lifespan.(Liang & Bushman, 2021)

Bacteriophages or Phage

Bacteriophages, or phages, are viruses that infect and replicate via target bacterial cells and are the most abundant viruses found throughout the body with the largest population and diversity found within the gut bacteriome. The replication dynamics of these bacteriophages is dependent on the diversity and population of the bacterial cells found within the gut microbiota. (Figure 6)

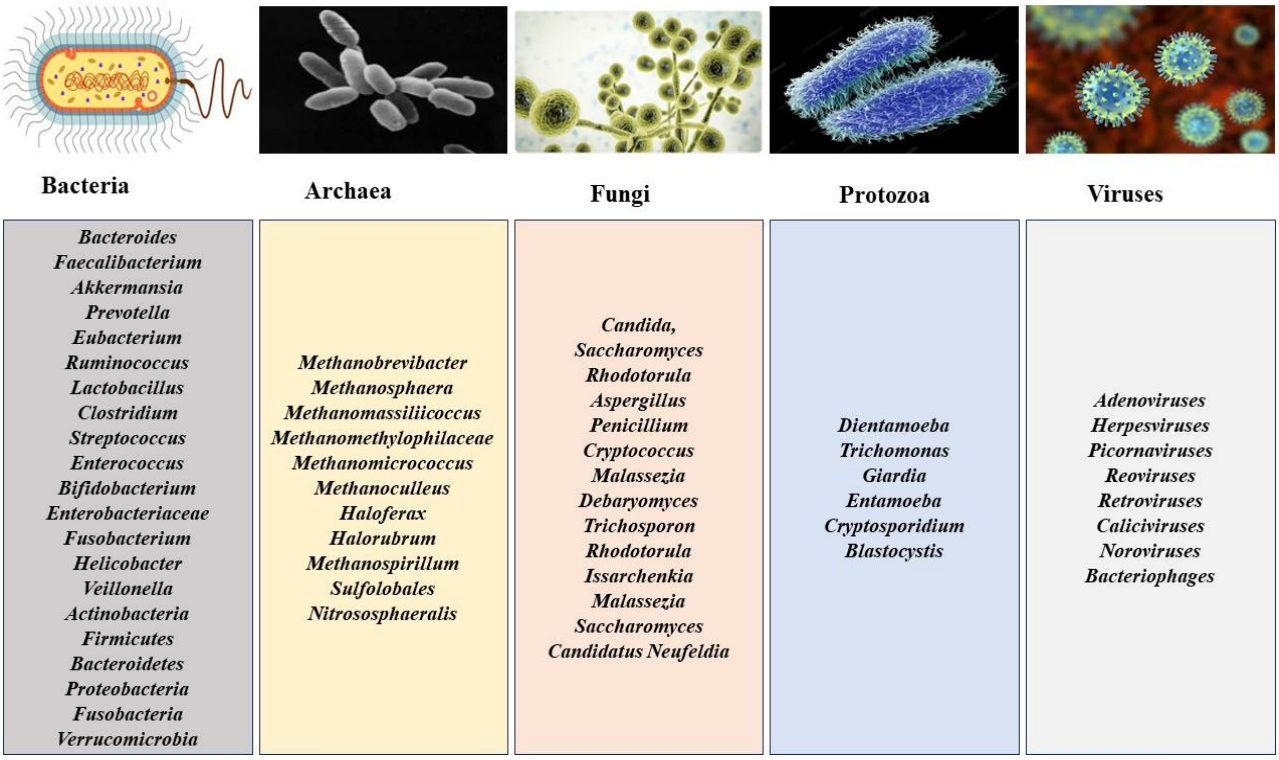


Figure 6. Microorganisms comprising the gut microbiome. (Emencheta et al. 2023)

Two of the largest specialized gut virome datasets are the "Human Gut Virome Database, GVD," which encompasses 13,203 viral populations, and the "Gut Phage Database, GPD," which encompasses 142,809 phages. (10)

The Gut Virome Database identifies, 97.7% of viral populations are bacterial viruses (bacteriophages), 2.1% are eukaryotic viruses, and 0.1% are archaeal viruses.(Fig.6)

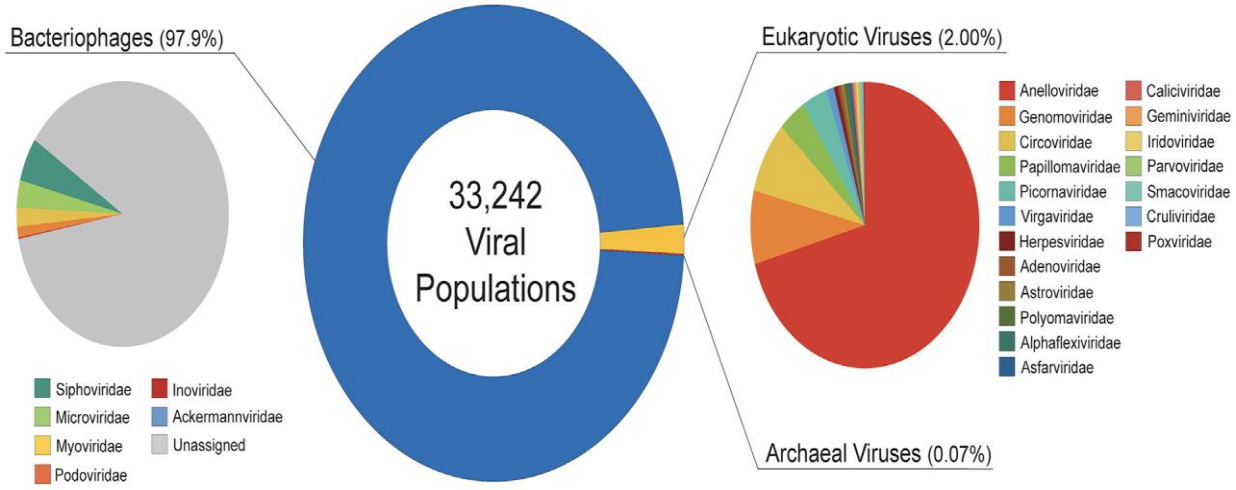


Figure 7. The Gut Virome Database,(Gregoy et al.2020)

(A) Pie charts showing the number of bacteriophages, eukaryotic viruses, and archaeal viruses in the GVD (centre) and their familial taxonomic composition by the bacteriophages (left) and the eukaryotic viruses (right).

Other recent efforts have produced 189,680 viral metagenome-assembled genomes (MAGs), and the latest version of IMG/VR contains over 15 million MAGs. Unfortunately, the reliability of these viral MAGs is uncertain and as such they are often not included in the popular reference databases, such as GenBank or RefSeq, which are typically used for annotating metagenomes. Furthermore, GenBank contains only 4,509 phage genomes (National Center for Biotechnology Information (NCBI), 1988), and these are prone to mislabelling.(8)

Bacteriophages were originally grouped into four basic morphological groups, i.e., tailed (*Caudovirales*), polyhedral *(Microviridae*), filamentous *(Inoviridae),* and pleomorphic *(Plasmaviridae*). With the advanced technology and next generation sequencing, bacteriophages are classified according to morphology, genomic properties, and life cycle. (9,10) (Table 3)

All vary in size, morphology, and genetics, but the protection of their nucleic acid genome protected by an outer shell of proteins is consistent across all the bacteriophages.(10) Genetically, they include DNA phages which include double-stranded DNA (dsDNA)-based genome phages or single-stranded DNA (ssDNA)-based genome phages. RNA phages are also grouped into double-stranded RNA (dsRNA)-based genome phages or single-stranded RNA (ssRNA)-based genome phages. A specific group of RNA Phages that are called the *retroviruses,* can convert their RNA genome into DNA via reverse-transcriptase enzymes. (Figure 8)

Table 3. Bacteriophage Classifications can be performed based on three significant characteristics: morphology, genomic properties, and life cycle. (Emencheta et al. 2023)

Morphology	**Genomic Properties**	**Life Cycle**
Caudovirales - Myoviridae - Siphoviridae	DNA Phages -Double stranded DNA (dsDNA) -Single stranded DNA (ssDNA)	Temperate
Filamentous	RNA Phages -Double stranded RNA (dsRNA) -Single stranded RNA (ssRNA)	Lytic

Tectiviridae Inoviridae Leviviridae Microviridae Pleolipoviridae	Retroviruses Circular Replicating Phages Temperate Phages Virulent Phages	Lysogenic

Bacteriophages interact with their bacterial cells through four different ways. Bacteriophages infect the bacteria, create viral macromolecules, assemble new particles, and lyse bacterial cells to release new viral particles during the lytic proliferation. During lysogenic growth, phage genomes are injected into bacterial cells which incorporate into the bacterial cellular chromosome. These cells remain in a quiescent state until an appropriate induction signal is detected, and the phage genome is excised and goes on to lytic growth.(4)(Figure 9).

With pseudo-lysogenic bacteriophages or temperate bacteriophages there is a loose contact between the phage and the host where the phage genome is present in the within the bacterial cell but is not actively driving any lytic development. Finally, some phages, like the filamentous phages (*Inoviridae),* have the ability to infect bacterial cells, produce new phage progeny but preserving the host bacterial cell.(3,4)(Figure 10)

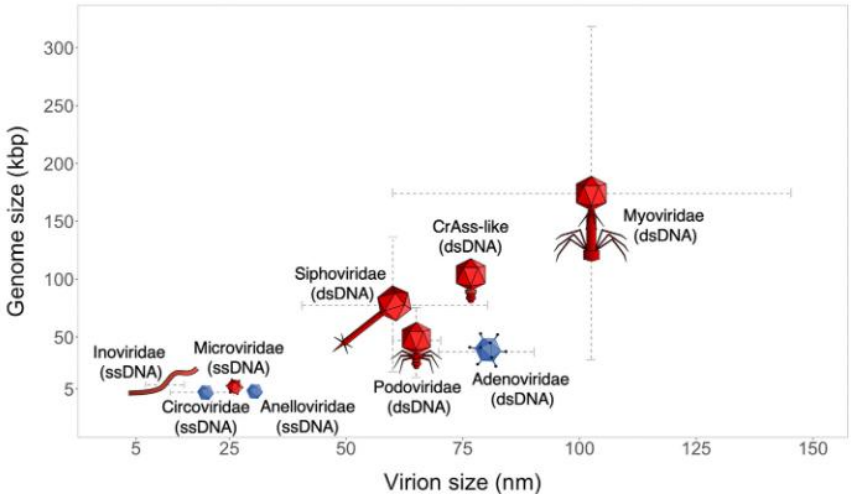


Figure 8 Size distributions of genomes and virions of the most prevalent virus families in the gut. (Garmaeva et al, 2019)

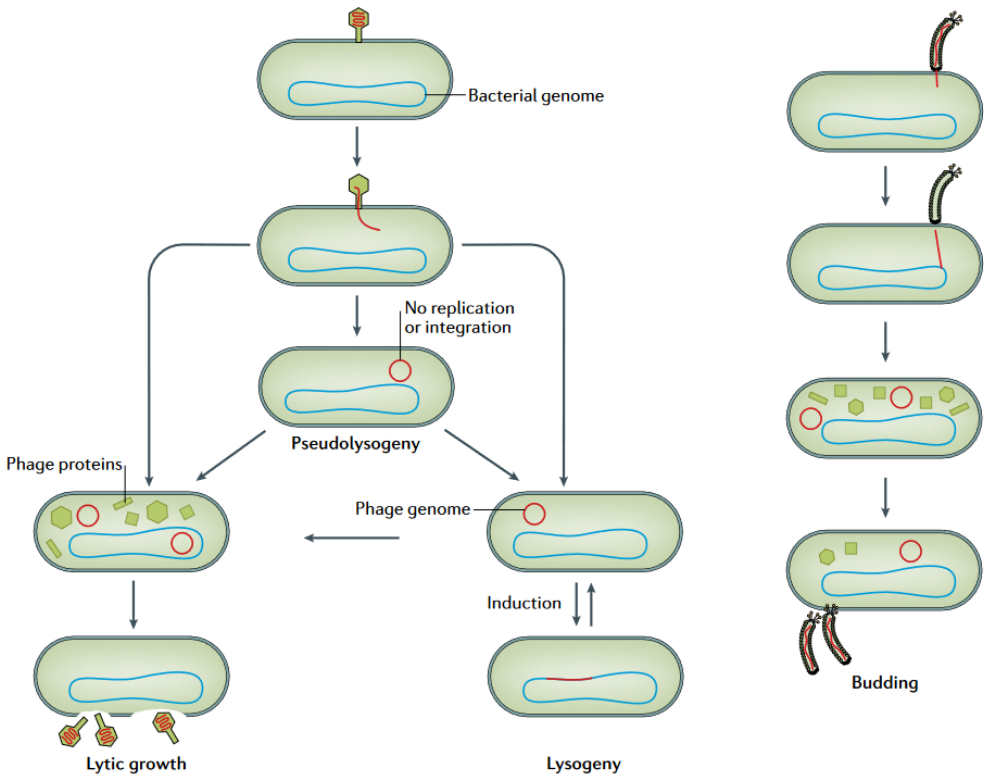


Figure 9.Phage replication cycles.(Liang et al,2021)

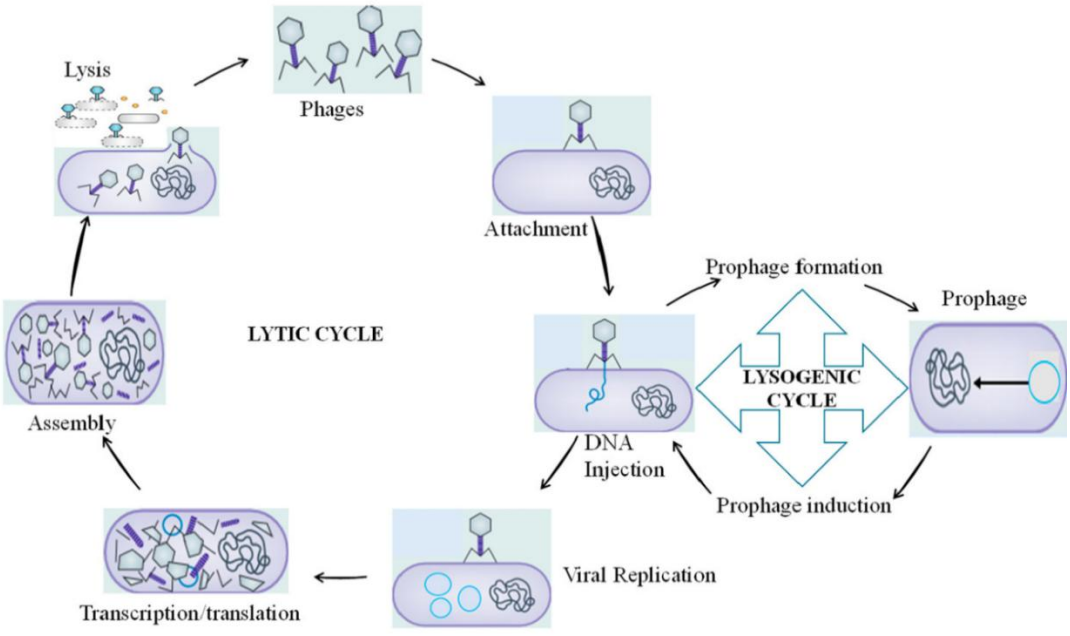


Figure 10. The Lytic or Lysogenic pathways of a phage lifecycle.(Emencheta et all.2023)

Most observed bacteriophages carry the prophage genes, and these lysogenic prophages are proposed to follow the piggyback-the-winner (PtW) dynamic, which is often seen in high virus-microbe-ratio (VMR) environments.(14)

Lytic phages, follow the traditional kill-the-winner (KtW) viral dynamic, seen commonly in lower VMR environments. Both PtW and KtW are seen in the human gut simultaneously likely due to the variety of phage species that exist and the hosts they infect being present at varying levels. Multiple external signals, including antibiotics, Fe2+ levels, and DNA damage, can influence the bacteriophages by either switching on the lysogenic pathway or the lytic pathway.(10)

In line with this, several mechanisms for the regulation of gut bacteria by bacteriophages have been proposed: “Kill the Winner”, “Biological Weapon”, “Community Shuffling”, and “Emerging New Bacterial Strain”.(14,15)

In the “Kill the Winner” theory, the fastest proliferating gut bacteria are usually targeted by the bacteriophages, lowering their numbers. However, the evidence for this being a common occurrence is limited, with research showing that it only happens for a few dominant bacteria.

The “Biological Weapon” model meanwhile theorizes that bacteriophages are utilized by gut commensal bacteria to kill other competing bacteria for the intestinal environment. This may also be protective against bacterial pathogens, however, there is a dearth of supporting experimental evidence. (14,15)

In the “Community Shuffling” model, an environmental stressor or a host reaction, such as antibiotic therapy or inflammation, may induce bacteriophages to infect bacteria, leading to lysis. This may lead to displacement or reduction of commensal bacteria which results to intestinal dysbiosis. For instance, antibiotic drugs can induce this phenomenon in *Escherichia coli*, *Clostridium difficile*, *Enterococcus faecalis*, or *Staphylococcus aureus*.(8,9)

Bacteriophages can also transfer genes to bacteria without killing them through the lysogenic life cycle, which is seen in the “Emerging New Bacterial Strain” theory. This makes the bacteria function as reservoirs of genetic diversity. For example, studies have shown that *Enterococcus faecalis* has a high degree of prevalence and genomic variation in the mammalian gastrointestinal tract. It is postulated that this genomic variation can be due to the introduction of bacteriophage genes into the bacterial genome.(8,9) (Figure 11),(Table 4)

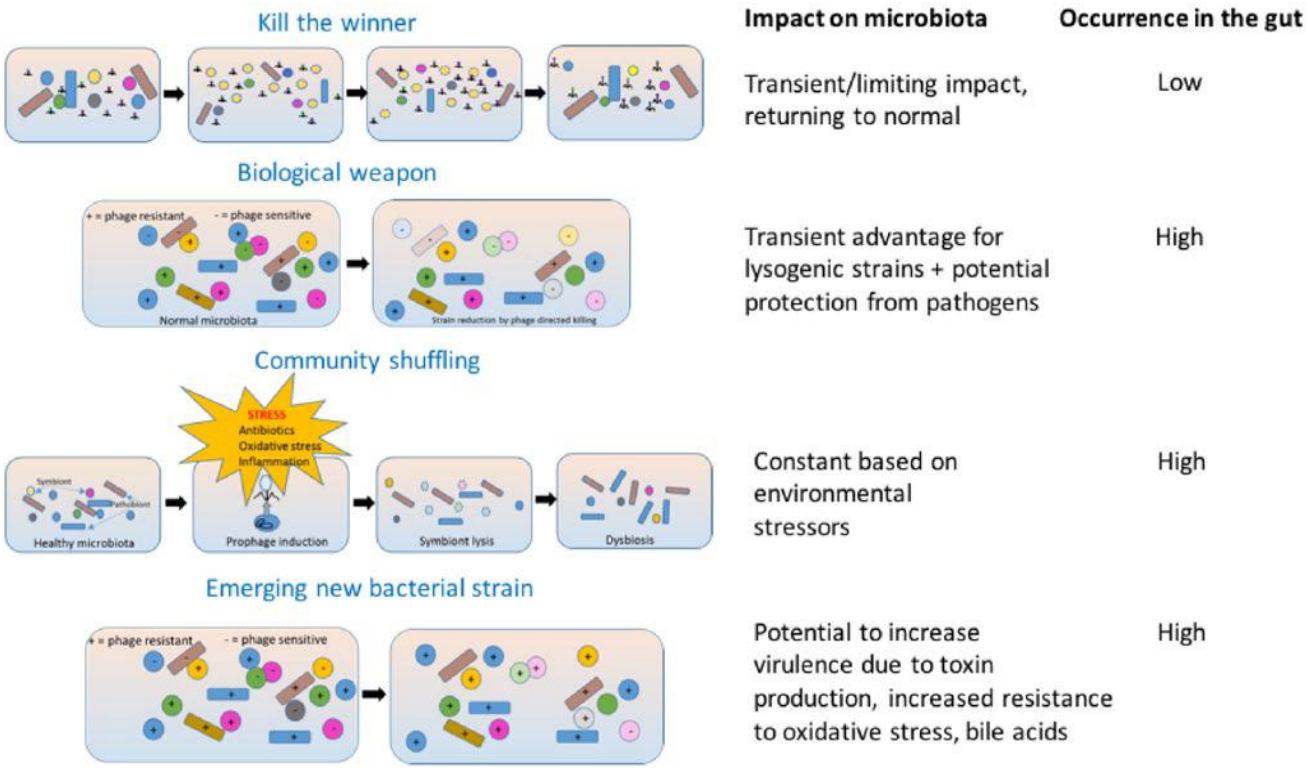


Figure 11. Proposed mechanisms for the regulation of Gut Bacteria by bacteriophages. (Mukhopadhya et al.2019)

Table 4. Summary of viral populations of the gut virome and models of interaction with the human host. (Columpsi et al. 2016).

Model of interaction	**Supposed mechanisms**
Kill the winner	Bacteriophages act as predators of overgrown bacteria
Biological weapon	Bacteriophages are used by commensal bacteria to kill bacterial competitor for the intestinal environment
Community shuffling	A host reaction induced bacteriophages to act negatively on their host
Emergence of new bacterial strains	Bacteriophages operate as reservoirs of genetic diversity, without killing bacteria

Global assessment of the human virome associated a distinct group of host bacteria namely *Bacteroides*, *Prevotella* and *Ruminococcus* with their respective virome. (10) The healthy human gut virome core is colonized with almost 95% from members of the *Caudovirales (dsDNA)* (the

crAssphages—cross assembly phages), and *Microviridae* and the rest from *Geminiviridae, Herpesviridae, Nanoviridae, Papillomaviridae, Parvoviridae, Polyomaviridae, Adenoviridae and Circoviridae.* (Table 5)

This was attributed to the early colonization of the gut by *Bacteroides*, *Proteobacteria* and *Actinobacteria* because CrAss-like phages are known to infect bacterial species belonging to the abundant *Bacteroides genus B. intestinalis,* and *Bacteroides xylanisolvens.*(10)

CrAssphages exhibit a worldwide distribution and are most frequently detected in over 50% of human gut content samples. A human gut viral population may contain up to 90% crAssphages.

Table 5. Virus communities within the human gut.(Mukhopadhya et al,2019)

Gut bacteriophages	
Mostly double-stranded and single-stranded DNA phages:	
Myoviridae, Podoviridae, Siphoviridae, Inoviridae and Microviridae	
DNA viruses:	
Double-stranded	*Single-stranded*
Adenoviridae	Anelloviridae
Herpesviridae	Circoviridae
Iridoviridae	
Marseilleviridae	
Mimiviridae	
Papillomaviridae	
Polyomaviridae	
Poxviridae	
RNA viruses	
Double-stranded	*Single-stranded*
Picobimaviridae	Caliciviridae
Reoviridae	Astroviridae
	Virgaviridae
	Picornaviridae
	Retroviridae
	Togaviridae
Definitive pathogenic eukaryotic viruses infecting the gut	
Rotavirus, norovirus, astrovirus, adenovirus (serotypes 40 and 41), enterovirus (only adenovirus is DNA virus, rest are all RNA viruses).	

Eukaryotic Viruses

Eukaryotic viruses can cause one of four different outcomes for their host cell. The most common outcome is host cell lysis, resulting from a virulent infection (essentially the lytic cycle of replication seen in phage). Some viruses can cause a latent infection, co-existing peacefully with their host cells for years (much like a temperate phage during lysogeny). Some enveloped eukaryotic viruses can also be released one at a time from an infected host cell, in a type of budding process, causing a persistent infection. Lastly, certain eukaryotic viruses can cause the host cell to transform into a malignant or cancerous cell, a mechanism known as transformation.(3)

The healthy human gut usually has low populations of eukaryotic viruses, which are dominated by the pathogenic viruses such as Enterovirus, Rotavirus, Astrovirus and Norovirus. These are known to be causative agents of infections such as gastroenteritis, but they have also been studied to be commensal inhabitants of the gut providing essential immunity and gut homeostasis.(3)

In the steady state, RNA virus can be recognised by retinoic acid inducible gene-1 (RIG-I) receptor of dendritic cells (DCs) to initiate the synthesis of interleukin-15 (IL-15) to preserve intraepithelial lymphocyte (IEL) balance maintain the homeostasis of IELs. (3) (Figure 12)

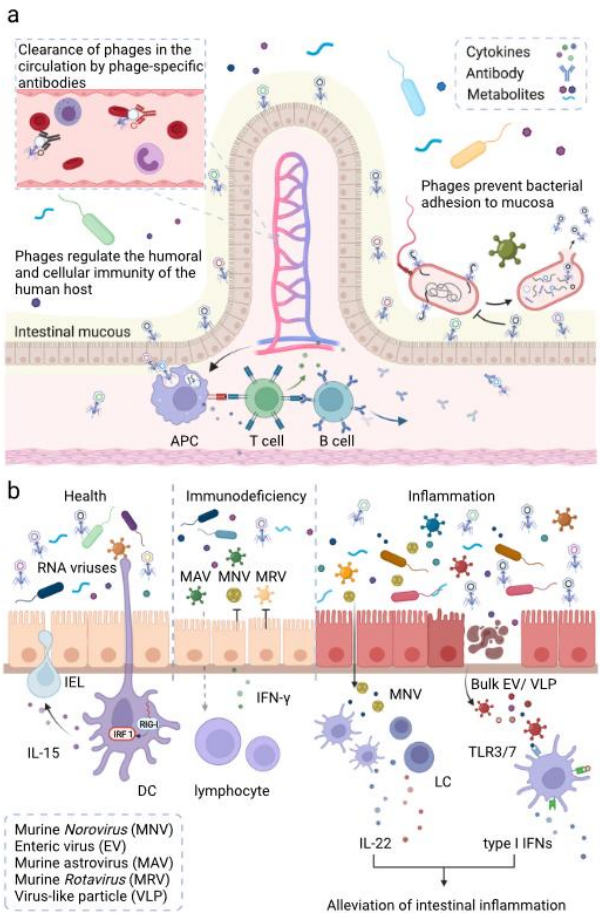


Figure 12 Gut virome and host immunity.(Cao et al,2022)

Animal studies have shown that Murine Rotaviruses (MRVs) reduce intestinal damage in mice by activating IFN-I/IL-22-dependent pathways during the inflammatory state. By producing IFN-g, astrovirus supplementation can shield mice from viral (MNVs/MRVs) infection when they are immunodeficient.(1,3)

Archaeal Viruses

Archaea are natural inhabitants of the gut microbiota. Because they flourish in harsh conditions (high pressure, high temperature, and high pH), these unicellular microorganisms have been dubbed "extreme bacteria."(12,16) A distinctive metabolism known as "methanogenesis" is present in certain archaea. Methanogenic archaea are strict anaerobes found in soils, freshwater and marine sediments, and the digestive tracts of humans and animals.(12)

Methanogenic archaea have been identified as inhabitants of the human gut for over 30 years due to the isolation of two methanogenic species, *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* (both of which belong to the order *Methanobacteriales*), and the detection of methane in breath. The most frequent methanogenic colonizer in humans is M. smithii, which is followed by *M. stadtmanae. M. smithii* settles in from the cecum to the rectum. Flatus or burping are body's natural mechanisms to evacuate the methane produced.(16,33)

Archaeal methanogens through their metabolic activities deplete Trimethylamine, TMA levels providing beneficial and positive on the host's health. Intestinal microbiota metabolize choline, lecithin, L-carnitine, and foods rich in TMA for example fish and synthesize TMA.

TMA is subsequently oxidized in the liver to produce Trimethylamine N-oxide, TMAO, which is eliminated via urine. Increased levels of TMAO is seen to contribute to platelet hyperreactivity and thrombosis and risk of atherosclerosis and cardiovascular diseases.(32,33). Archaeal methanogens metabolize TMA into methane; thus, less TMA is oxidised in the liver lowering plasma concentrations of TMAO. This reduction of TMAO provides a protective mechanism against TMAO-mediated cardiovascular diseases. (16,34).

Alterations in commensal archaea have been associated with irritable bowel disease, IBD, ulcerative colitis, UC and crohns disease, CD. *M stadmanae* is a strong inflammatory stimulator and its increase has been linked to the characteristic inflammation in IBD patients. (16,27,34).

Additionally, a lower *M. smithii* load may lead to a rise in gut sulfate-reducing bacteria, which can generate harmful hydrogen sulfide and contribute to the pathophysiology of IBD. (16,26,)

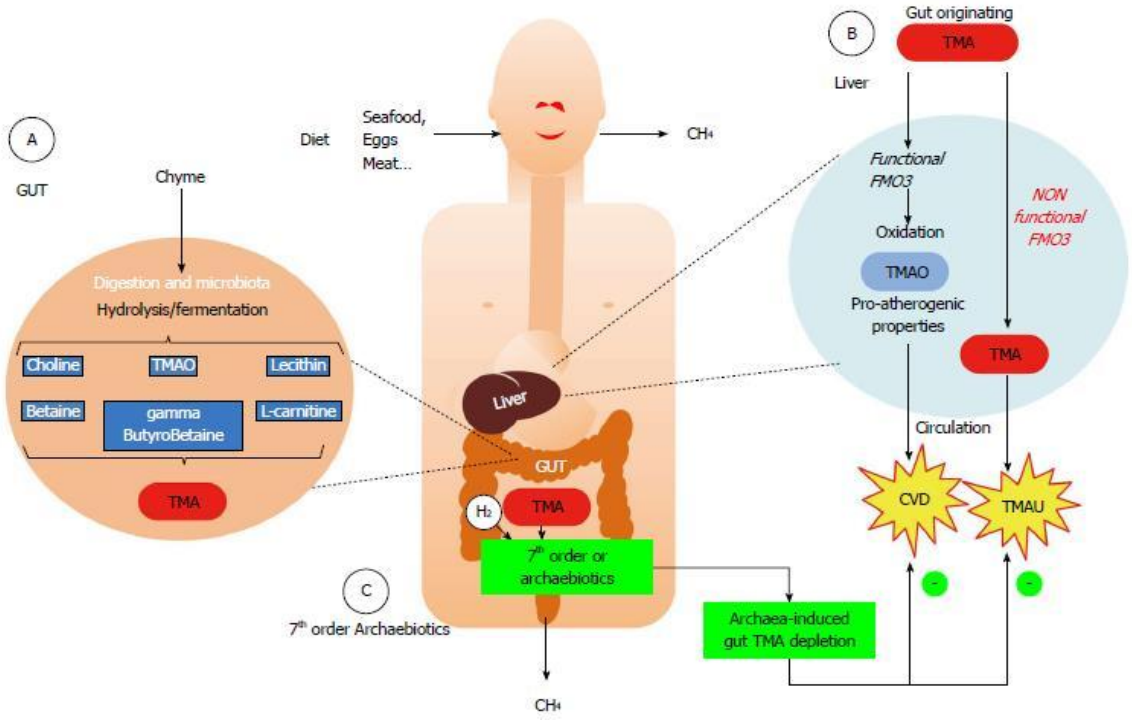


Figure 13 Faith of trimethylamine originating from the gut microbiota metabolism. (Ishac et al, 2015)

The American Society for Microbiology and Federation of European Biochemical Societies, FEBS dub The Archaeome: An Emerging Player in Health and Disease. (Fig.11) Methane production was directly correlated with constipation but inversely correlated with diarrhea in chemotherapy patients and it was elevated in patients with colorectal cancer and precancerous symptoms.

In chemotherapy patients, pH was negatively correlated with diarrhea but directly correlated with constipation. It has not been demonstrated that methane causes cancer, however methane oxidation produces the carcinogenic formaldehyde. (6,16)

Lower methanogenic archaea levels cause less hydrogen to be converted to methane, which increases the generation of hydrogen sulfite by sulfate-reducing bacteria and raises the risk of colonic epithelial cell damage that could result in colorectal cancer. (16,23)

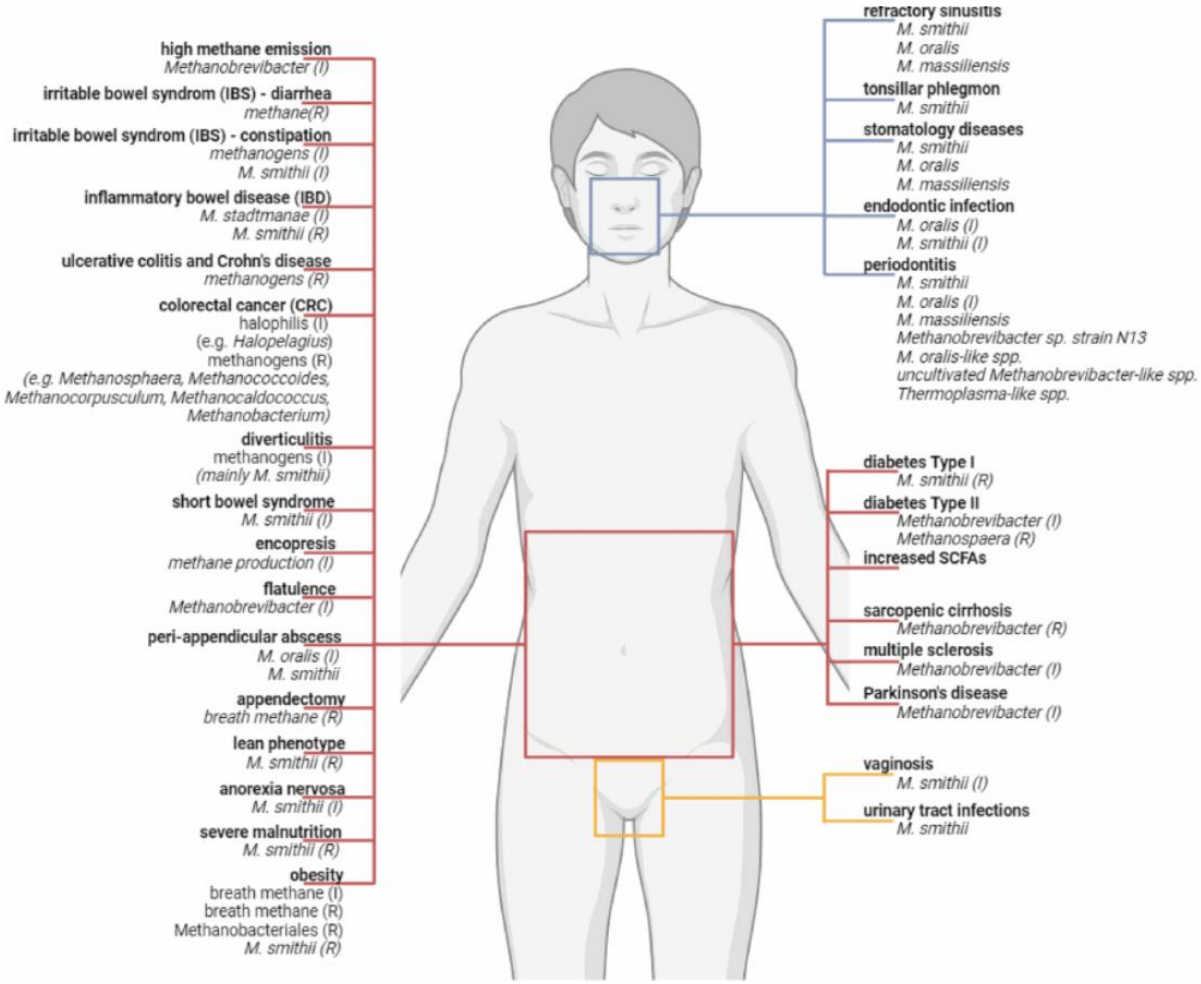


Figure 14 Conditions associated with altered archaeal community composition.(The FEBS Journal, 2024.

CHAPTER 3: Function of the Gut Virome and Interkingdom interactions

The diversity of the gut virome plays an important role in maintaining the balance between health and disease by modulating the immune system. It regulates intestinal homeostasis and inflammation by causing continuous low-level immune response without any apparent symptoms.(3,36)

The gut virome achieves this by interacting with other components of the gut microbiome and the host's genetics. Bacteriophages influence the composition of the bacteriome by infecting them, and the gut bacteriome may in turn, regulate the gut virome and its replication dynamics.(10)

Recent research on animals and in vitro suggests that phages may have direct interactions with the host immune system. Without the help of bacteria, phages can be absorbed by immune cells and use Toll-like receptor (TLR) signalling to initiate immunological responses. (3,21)

This work also showed that phages from *Lactobacillus, Escherichia,* and *Bacteroides* can activate the nucleotide-sensing receptor TLR9 to produce IL-12, IL-6, IL-10, and IFNγ. All things considered, the interactions between bacteria, phages, and the host immune system probably play significant roles in maintaining host immunological homeostasis.(21)

The majority of resident viruses, including phages and human viruses, are not enveloped, which is a noteworthy feature among gut viruses. This makes sense because lipid envelopes are unlikely to withstand the bile salts' detergent effects, the large intestine's dehydration, and the external environmental conditions necessary for transmission over the fecal–oral route.(3,21)

Interactions between the Bacteriophage and Microbiota in the Gut

As already mentioned , the bacteriophages are the most numerous members of the gut virome. For this reason, the interactions between bacteriophages and the gut microbiota in a Healthy individual may have a profound effect on the bacterial community living in the gut. The ratio of virome types to species-level bacterial phylotypes is almost 1:1 and their replication dynamics are closely tied to bacterial populations and their replication.(3,4,36)

Before the advent of tools available for virome study, little was known in the field as only direct observation with the counting of virus-like particle (VLP) methods with epi-fluorescence and transmission electron microscopy was used for their investigation.(4)

These microscope-based techniques were used to quantify the total counts of bacteriophages in human faecal samples, colonic mucosa, and caecal components, taking into account the large number of viral morphotypes per individual. These were estimated to be between 109–1010 VLPs per gram, revealing the *Caudovirales* order, represented by *Myoviridae*, *Podoviridae*, and *Siphoviridae* as the most frequent.

However, because many of the bacteria in the distal gut, including *Ruminococcaceae* and *Lachnospiraceae*, are difficult to control or cultivate in the lab, these techniques do not accurately characterize the gut virome. Therefore, the actual diversity of human gut bacteriophages is not reflected in the collections of phage strains of human faecal samples that are currently available.(36)

More recently, new technologies such as high throughput metagenomic sequencing technology have made it easier to see how diverse and abundant human gut bacteriophage populations actually are.(4,36)

The balance between the phage's lytic and lysogenic life cycles can impact this homeostasis and significantly affect human health as phages have the ability to transfer DNA between cells, giving bacterial genomes new capabilities that could alter their virulence and fitness.(6,23)

(Figure 9,Figure 10)

Individual differences in intestinal virome diversity are demonstrated by mono-and dizygotic twin studies and they are associated with the diversity of the gut microbiota as a whole.(36) As prophages, phages often lead a temperate existence and integrate into their bacterial hosts. The lytic cycle, which results in viral multiplication and, eventually, host cell death, can be triggered by environmental stresses. Prophages can influence the dynamics of the gut ecosystem by contributing to genetic factors like virulence factors or genes that resist antibiotics. IBD and colorectal cancer are two dysbiosis-related conditions that change the gut's phage population.(16)

Interactions between the Gut Virome and Host Immunity

There is now sufficient evidence to conclude that the gut virome, particularly the bacteriophages, can interact directly with the immune system by triggering innate and adaptive immune responses.(1) Studies have shown that phages taken orally go into systemic tissue in vivo and elicit both innate and adaptive immune responses there.(11)

One such instance is that bacteriophage-induced lysis create pathogen-associated molecular patterns (PAMPs) which can lead to a low degree of immune response without causing symptoms, leading to potential host resistance to other pathogens and susceptibility to diseases.(9)

The proliferation of bacteriophages is linked to an increase in gut virome richness and a decrease in gut bacterial variety and richness, a phenomenon known as "microbial dysbiosis," which may be the cause of persistent inflammation in IBD. Increased bacterial lysis and the production of microbe-associated molecular patterns (MAMPs), which may draw inflammatory cells to the lamina propria, are further consequences of altered viral–bacterial dynamics. The inflammatory cascade may potentially be the cause of the luminal alterations, which might be considered an "epiphenomenon." (8, 16).

Research has also shown that bacteriophages that adhered to gut mucosal surfaces can contribute to building an immune defence against possible bacterial pathogens by providing an antimicrobial barrier.(1) These may be termed as "long term passengers" or commensals.(16)

Since eukaryotic viral particles and bacteriophages can trigger innate immunity, research has attempted to comprehend the relationship between the gut virome and the immune system. The development of type IFN-α and -β as well as inflammatory cytokines (IL-1 and IL-6) is thought to be triggered by the detection of viral nucleic acids within cells using a variety of pattern-recognition sensors for RNA (RIG-I and Toll-like receptors TLR7 and TLR8) and DNA (TLR9, cyclic-GMP-AMP). This innate immune activation may offer protection against pathogenic viral infections.(3,16)

Given that phages predominate over eukaryotic viruses, it is possible that phages are the primary cause of this activation. Nevertheless, eukaryotic viruses are also known to have positive impact because TLR3 and TLR7 preferred intestinal homeostasis via anti-inflammatory cytokines.(3,16)

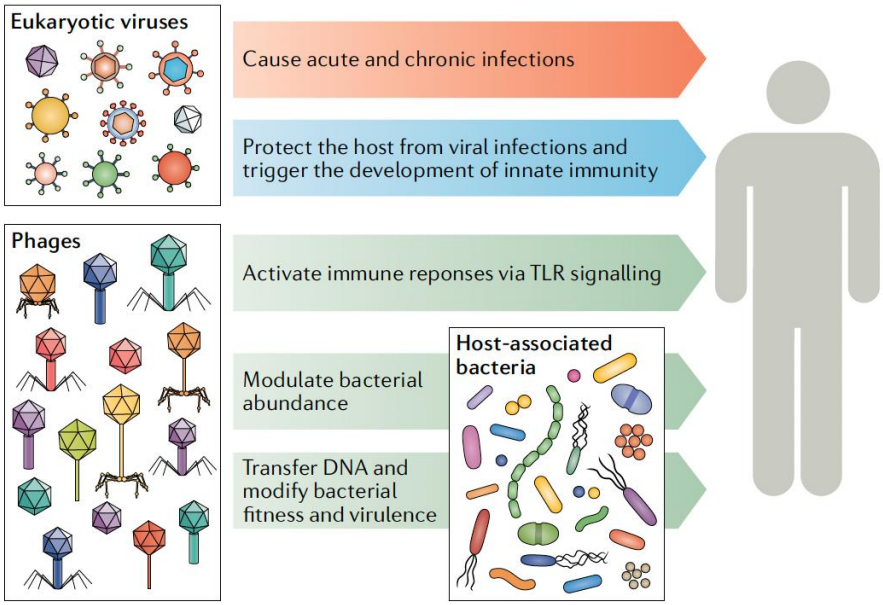


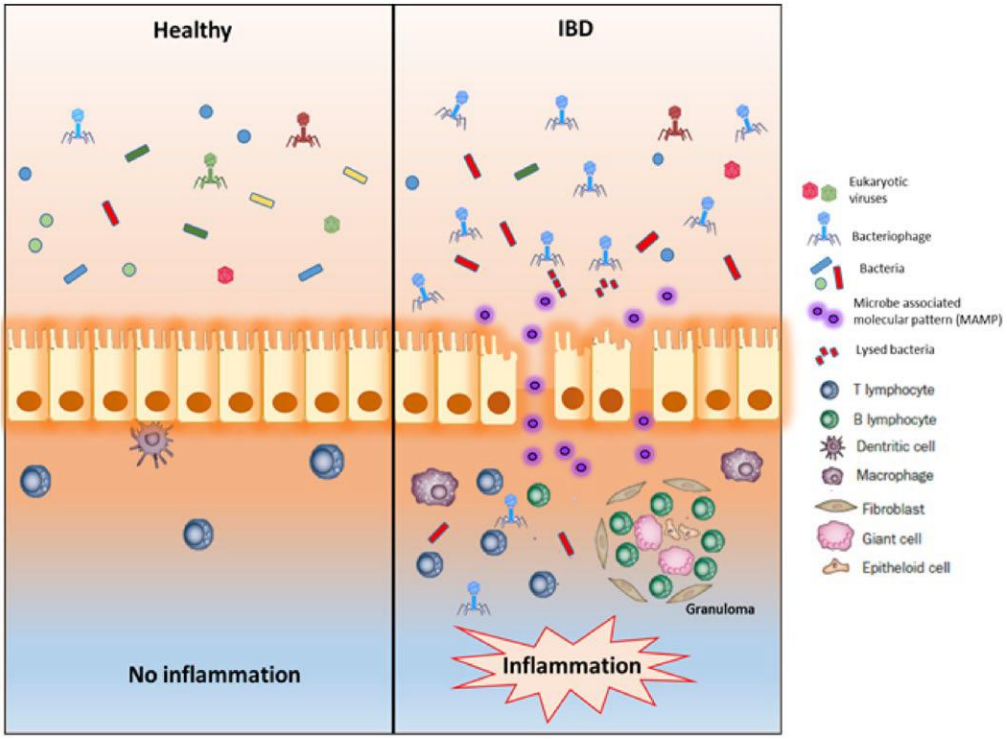
Figure 15 Eukaryotic viruses can affect host health in both positive (blue arrow) and negative (red arrow) ways. Phages have unknown effects (green arrow) on host health and can interact with the host directly or indirectly through the host-associated bacterial flora. (Liang & Bushman,2021)

Eukaryotic viruses can affect host health in both positive (blue arrow) and negative (red arrow) ways. Phages have unknown effects (green arrow) on host health and can interact with the host directly or indirectly through the host-associated bacterial flora. (Liang & Bushman, 2021).

Recently, researchers focused on a study that demonstrated that intestine intraepithelial lymphocytes (IELs) require commensal viruses to maintain homeostasis. IELs have important functions in defending the gut mucosa. This work suggests that commensal viruses have comparable but distinct functions in maintaining intestinal homeostasis, highlighting the need of include them in studies on intestinal health and illness.(1,11)

Several pro-inflammatory cytokines are released as a result of the activated receptors' activation of phagocytosis, respiratory burst, and intracellular signalling pathways. The intestinal mucosa becomes inflamed as a result of the subsequent production of the pro-inflammatory cytokines IL-Iβ, TNF-α, INF-γ, IL-6, IL-17A, and IL-23, which may be the underlying pathophysiology of UC and CD.(32,33)

The bacteriophages and bacteria's evolutionary survival strategies create a dynamic and never-ending arms race, sometimes known as the Red Queen race or hypothesis, in which both sides are "continuously running to stay in the same place" or evolving equally but keeping up with one another, creating a zero-sum game.(11)



➢ Balance between virome and bacteriome	➢ Altered dynamics between virome and bacteriome
➢ No luminal trigger	➢ ↑Viral richness
➢ No inflammation	➢ ↓ Bacterial richness and diversity
	➢ Chronic inflammation in response to:
	-Bacterial lysis and release of MAMPS -Altered virome -Altered dysbiotic bacteria

Figure 16 Diagrammatic illustration of how inflammatory bowel illness alters the bacteriome and enteric virome.(Mukhopadhya et al, 2019)

CHAPTER 4: Alteration of the Gut Virome and its Association with Gastrointestinal diseases and Extra Gastrointestinal symptoms

Through inflammatory pathways, intestinal bacteriophages may play a role in the shift from health to disease by creating an imbalance between the microbiome and host symbiotic bacteria. This dysbiosis of symbiotic bacteria and bacteriophage lead to intestinal and extra-intestinal disease. (12,17,18)

An imbalance such as an increase in the numbers of the disease-causing *Caudovirales* and a reduction in *Microvirales* can lead to inflammatory bowel disease such as ulcerative colitis and Crohn's disease.(12)

Patients with ulcerative colitis showed an increased number of *Caudovirales* (especially *Escherichia* and *Enterobacteri*a bacteriophages) in the inflamed gastric mucosa. These bacteriophages were shown to induce the gut mucosal immune response leading to subsequent inflammation.(8,16)

Eukaryotic viruses such as the *Retroviridae, Herpesviridae, Hepadnaviridae,* and *Hepeviridae* were also studied to exist in greater numbers in ulcerative colitis and Crohn's disease. Conversely, *Virgaviridae* was found to be reduced in both of those diseases.(13) (

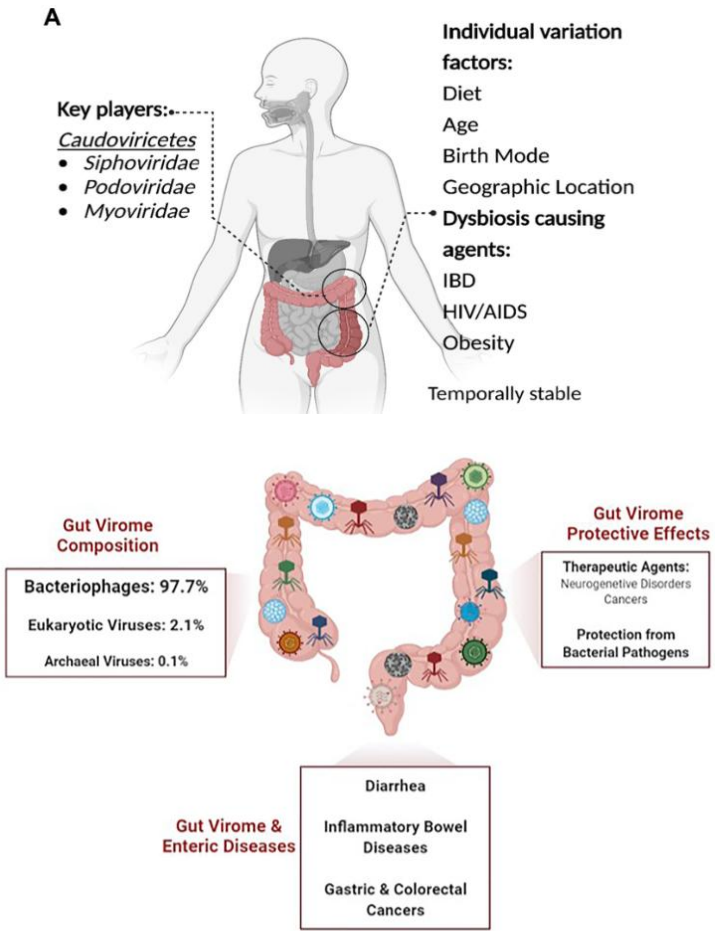


Figure 17 Above – Dysbiosis-causing agents and individual variation factors. (Pargin et al. 2023). Below - A diagram showing the gut virome composition, its protective effects, and its associated enteric diseases. (Spencer et al. 2022)

Table 6)

Regarding the bacteriophage and the bacterial population, four possible outcomes exist, which may indicate illness symptoms or stable equilibrium states preceding inflammation. If the bacteriophage component's richness rises in tandem with the bacterial richness, this would indicate that the latter are multiplying only because there are more host bacteria available for prey.

On the other hand, a decline in both elements can be seen as a lack of bacterial prey negatively affecting the bacteriophages. A rise in bacteriophage richness accompanied by a fall in bacterial richness, however, has a completely different meaning because it suggests that the former is the one causing and directing these changes. IBD patients have been shown to exhibit this pattern of alterations in the as documented in the seminal paper by Norman and Colleagues.(30,37)

According to the "biological weapon" paradigm, commensal bacteria would eliminate a rival bacterium for the intestinal environment by using their phages. The phage would give its carrier bacteria immunity against future infections in this situation. They would act as "biological weapons," causing a shift in the population's composition, dysbiosis, and, in certain situations, an inflammatory reaction by causing a large lysis of competing bacteria.(11)

According to the so-called "community shuffling" paradigm, prophage production in a number of bacterial species, including Clostridium difficile and E. coli, has been linked to stressors such oxidative stress, inflammation, and antibiotic therapy. The 30-fold increase in virus-like particles observed in biopsy specimens from Crohn's disease patients compared to healthy controls lends credence to this notion. By changing the interaction between bacterial symbionts and pathobionts, this prophage induction would exacerbate intestinal dysbiosis according to the "community shuffling" hypothesis.(11,41)(

Figure 17)

Virome-induced intestinal dysbiosis is now regarded as a contributing factor to Crohn's disease (CD), inflammatory bowel disease (IBD), and colon cancer. This was further supported when up to 70% of people with IBD had cytomegalovirus (CMV), and reactivation of this virus may be linked to a kind of colitis that exhibits some IBD symptoms. It was demonstrated that antiviral treatment for CMV in

IBD patients has no appreciable effect on the course of the inflammatory disease, even if viral elements from CMV may be involved. This further provides credence to the idea of virome-induced intestinal dysbiosis.(11,38). The bacteriophages were further identified by epifluorescence microscopy in mucosal samples of patients with CD compared to controls and was confirmed by DNA sequencing.(8,11,12)

Additionally, people with obesity and type I and type II diabetes have significant alterations in their gut virome, particularly the phageome.(14,30). Fecal virome transplantation (FVT) reduced the symptoms and modified the gut microbiome and virome, delaying the formation of obesity in C57BL/6 laboratory mouse strain given high-fat diets. A change in the viral population from virulent to temporal phages or the restoration of lost phages that regulate the microbial communities could be the cause of this. (14,23)

Several pro-inflammatory cytokines are released as a result of the activated receptors' activation of phagocytosis, respiratory burst, and intracellular signalling pathways. The intestinal mucosa becomes inflamed as a result of the subsequent production of the pro-inflammatory cytokines IL-Iβ, TNF-α, INF-γ, IL-6, IL-17A, and IL-23, which may be the underlying pathophysiology of UC and CD.(32,33)

Gut microbiomes of 647 one-year-old children from the Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC2010) mother-child cohort was examined. All the children were extensively phenotyped at birth and had asthma diagnoses that were evaluated over time. It was discovered that certain temperate gut phage taxa were linked to the later onset of asthma. Specifically, it was discovered that this connection was substantially influenced by the combined abundances of 19 caudoviral families. An independent virome-asthma correlation was suggested by the cumulative impact on asthma risk of combining the bacteriome and virome signatures linked to asthma. Furthermore, the host TLR9 rs187084 gene variant was shown to influence the virome-associated asthma risk, indicating a direct connection between the host immune system and phages.(28,39)

It has been demonstrated in an experimental model that bacteriophage transfer is driven by intestinal inflammation; in particular, transfer of the prophage SopEΦ was elevated in the presence of intestinal inflammation, with a lysogenic conversion to ATCC14028S of >55%, as opposed to a lysogenic conversion reduction of 105 in the absence of any inflammation between the two *Salmonella Typhimurium strains,* SL1344 and ATCC14028S. (8,11,38)

This was supported with both, ileal samples from control patients and colonic biopsies, gut washes, and ileal biopsies from paediatric CD patients found the same three members of the *Caudovirales*

family with Transmission Electron Microscope, TEM.(11,28) and these virome changes in CD patients have been validated by metagenomic surveys.(11,13,20)

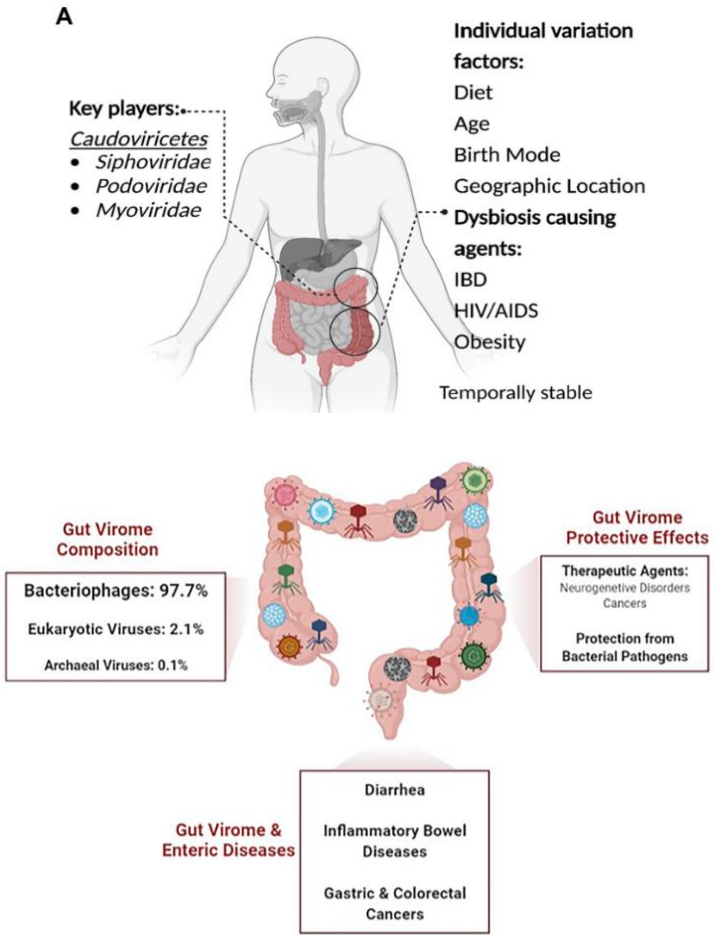


Figure 17 Above – Dysbiosis-causing agents and individual variation factors. (Pargin et al. 2023). Below - A diagram showing the gut virome composition, its protective effects, and its associated enteric diseases. (Spencer et al. 2022)

Table 6. Disease associated alterations in gut viral species. (Krishnamurthy et al. 2023; Spencer et al. 2022)

	Condition	Commensal bacteriophage	Commensal virus	Directionality
GI conditions	Ulcerative colitis	*Caudovirales*	*Pneumoviridae, Herpesviridae, Virgaviridae, Circoviridae, Picobirnaviridae, Orthopneumovirus*	↑
		Microviridae	*Anelloviridae, Polydnaviridae, Tymoviridae, Coccolithovirus, Minivirus,* Vertebrate-infecting virus *Orthopoxvirus*	↓
	Crohn's disease	*Caudovirales, Siphoviridae, Myoviridae, Podoviridae*	*Retroviridae, Herpesviridae, Hepadnaviridae, Hepeviridae*	↑
		Microviridae	*Virgaviridae*	↓
Metabolic conditions	Type 1 diabetes		*Enterovirus, Kobuvirus, Parechovirus*	↑
		Podoviridae, Myoviridae		↓
	Type 2 diabetes	*Siphoviridae, Podoviridae, Myoviridae, Caudovirales, Escherichia phage, Geobacillus phage, Lactobacillus phage*		↑
Liver disease	Non-alcoholic fatty liver disease		*Adenovirus*	↑
	Alcohol-associated liver disease	*Enterobacteria phages, Escherichia phages, Enterococcus phages*	*Parvoviridae, Herpesviridae*	↑
Cancer	Colorectal cancer	*Inovirus, Tunalikevirus*	*Herpesviridae, Cytomegalovirus, Epstein-Bar virus, Human papilloma virus, Polyomavirus, Orthobunyavirus*	↑
		Enterobacteria phages, crAssphages		↓

Diarrhoea has long been associated with Eukaryotic viruses in particular *Rotavirus, Noravirus,* however recent metagenomic studies have reported that novel commensal viruses may also be responsible for acute infections and diarrhoea. Bacteriophages and eukaryotic viruses such as *Anelloviridae, Adenoviridae, Caliciviridae, Astroviridae,* and *Reoviridae* were detected in samples from patients with acute diarrhoea.(3,28)

Diarrheal symptoms along with severe body pains and lack of taste were amongst the most common complaints from patients with SARS-Covid 2 infection. Bioinformatic analysis shows that Angiotensin – converting enzyme 2 is expressed in the upper eosophagus, and in absorptive enterocytes from ileum and colon.(24,28)

Patients with small bowel inflammation had decreased expression of Ace2 and therefore suffered severer Covid-19 infection symptoms including diarrhea, acute lung failure and cardiovascular complications because structural studies demonstrated that Ace2 has a significantly stronger binding affinity, 10-20 times more than its 2003 SARS-CoV-2 predecessor (24,28)

ACE2 is involved in the absorption of dietary amino acids controlling the expression of antimicrobial peptides and supporting the balance of the gut microbiome. Colitis was linked to ACE2 changes in mouse models, indicating that viral activity may modify enzymes, making the body more vulnerable to intestinal inflammation and diarrhea.(41)

Hashimoto and Colleagues conducted a molecular study in 2012 on Ace2 and reported mechanistically, ACE2 functions independently of RAS, controlling the ecology of the gut microbiome, the production of antimicrobial peptides, and intestinal amino acid balance. It was possible to transfer the increased risk of developing severe colitis onto germ-free wild-type mice by transplanting the modified microbiota from Ace2 mutant mice.(16)

Dietary tryptophan can directly influence ACE2-dependent alterations in gut microbiome and epithelial immunity. According to their findings, ACE2 is a crucial modulator of innate immunity, gut microbial ecology, transmissible susceptibility to colitis, and dietary amino acid balance. Their findings offer a mechanistic explanation for the connection between intestinal inflammation and diarrhea caused by amino acid deficiency.(16,42)

Tryptophan decarboxylase, the enzyme that converts tryptophan to tryptamine, is expressed by the commensals *Ruminococcus gnavus* and *Clostridium sporogenes*. Tryptamine is thought to increase GI motility by causing enterochromaffin cells to produce serotonin (5-HT)(Figure 18).Serotonin promotes gastrointestinal motility by activating serotonin type 3 receptors (5-HT3R).(14,42)

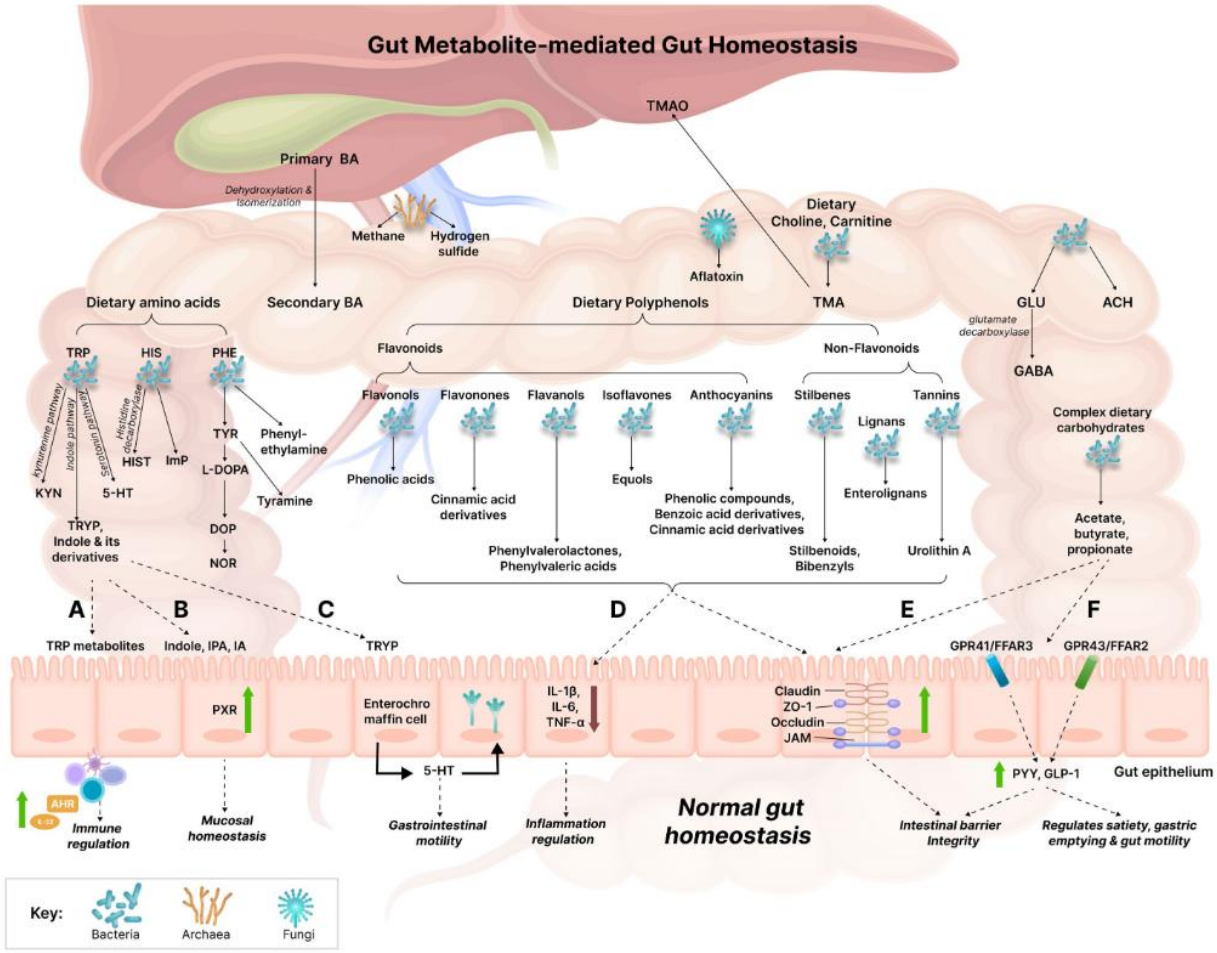


Figure 18 Gut metabolite-mediated gut homeostasis.(Krishnamurthy et al,2023)

Bacteria produce several molecules known as neurochemical interkingdom signalling neurohormones. The neurohormones produced are, short-chain fatty acids like gamma-amino butyrate which directly influence the release of the neurotransmitter serotonin from enterochromaffin cells and the release of acetylcholine and glucagon-like peptide −1 and peptide YY from enteroendocrine cells.(40)

The ENS neurons that innervate the intestinal mucosa may serve as the first neuronal detectors of signals from the microbiota, which subsequently alter vagal activity through synaptic transmission. Essentially, the gut-brain axis links hosts' emotion and cognitive behaviour's with microbial gastrointestinal residents, such as the virome, through interkingdom signalling between the gut bacteria and possibly the entire microbiome, nerve cell membrane vesicles, and the host endocrine, immune, and neural signalling pathways. (40,42)

A few bacterial species such as Streptococcus, Enterococcus, and Escherichia can synthesize serotonin, dopamine and norepinephrine, however the microbe-derived host responses are not well characterized. It has been proposed that gut dysbiosis of microbial species may cause aberrant immunological signals, an imbalance in host homeostasis, and the progression of diseases affecting the central nervous system.(14)

Bacteriophage affect bacteria in the gut through either lysogenic or lytic connections and following an infection, there is an increase in *Bacteroides* and a notable change in lipid metabolism, with an increase in sphingolipids, polyunsaturated fatty acids, and short-chain fatty acids.(14,40)

Antidepressants are among the most often prescribed medications for individuals with inflammatory bowel syndrome, and it is known that over 50% of IBD patients experience mental issues. The gut microbiome makeup changes in particular three bacterial genera, *Ruminococcus*, *Adlercreutzia*, and an unidentified genus in the order RF32 (class *Alphaproteobacteria*) that are impacted with the use of antidepressants, not forgetting patients with IBD already have an altered enteric virome (14,40,42)

CHAPTER 6: Therapeutic Potentials of the Gut Virome

Bacteriophage, or phage, therapy involves using bacteriophages to treat various infections. D'Herelle went on to carry out an in-depth study of these viruses, including replication and adaptation, and he proposed their possible use in anti-bacterial treatment. He called this area of research "bacteriophagy." When bacteriophages were discovered in 1915, they were administered to treat bacterial haemorrhagic dysentery, and shortly thereafter, commercial production of phage therapy became widespread. However, once antibiotics were formulated and mass produced, it led to the demise of bacteriophage therapy. Some of the downfalls of bacteriophage therapy was due to the fact that one phage cocktail did not work equally among all patients, and some patients had allergic inflammatory responses to the phage cocktail instead.(14)

Today, with the overuse of antibiotics has led to widespread antibiotic resistance and it is one of the biggest challenges faced globally amongst all healthcare institutes with a huge financial burden on the healthcare.(43)

Antimicrobials that lyse phage-infected microorganisms frequently cause the phages to transition from a lysogenic to a lytic lifestyle, killing their bacterial hosts and causing notable changes in phage abundance and proliferation as the diversity of bacteria decreases, while viral diversity initially increases as new viruses are released, before decreasing in response to the loss of their hosts.(14,41)

When antibiotics are administered for an extended period of time, oral and fecal virome react differently; in particular, oral virome is more varied than fecal virome. When blasted to the Comprehensive Antibiotic Resistance Database (CARD), the fecal virome had a higher abundance of homologous antibiotic resistance genes than the controls.(14,41)

These homologous genes target several known resistance pathways, such as multidrug transporters, tetracyclines, vancomycin, and beta-lactams. According to a different study examining the rise in phage antibiotic resistance genes after antibiotic medication, the rise in resistance genes begins on the third day of treatment. These research imply that the antibiotic resistance observed in a patient's microbial population may be influenced by the resistance indicators in bacteriophage.(14,41)

To combat this is through the use of bacteriophage therapy, as bacteriophages are host-specific, rather than broad spectrum.(14)Success with the use of Bacteriophage therapy was demonstrated recently, March 2025 where doctors and researchers from Duke-Nus and Singapore General Hospital used a cocktail of bacteriophage therapy to combat a very severe infection due to the culprit *Pseudomonas aeruginosa,* in a patient following surgery for her congenital heart condition.(43)

Another successful case report used T4-like bacteriophage therapy of an elderly patient who had developed necrotizing pancreatitis complicated by a pancreatic pseudocyst infected with multidrug-resistant *Acinetobacter baumanniii.* After five months of intracavitary and intravenous routes treatment, the patient recovered.(14)

Fecal Microbiota Transplantation (FMT) involves transferring a person's fecal microbiota to another person to increase the recipient's levels of "healthy" bacteria. FMT has been shown to be particularly successful in treating recurrent C. difficile infections and is frequently used as a treatment for gut dysbiosis.(14,41)

In Fecal Virome Transplantation, FVT trials, the given fecal material is filtered to separate the viruses, metabolites, proteins, and other minute particles for transplant from the bacteria and bigger components. Research has demonstrated that FVT and FMT both effectively treat C. difficile infections, and that phages are positively correlated with treatment success.(10,19,41)

As reported earlier, FVTs have successfully reduced the symptoms of type 2 diabetes in mice, reducing weight gain and changing the microbial community. They have also assisted in reestablishing the microbiome after antibiotics disrupted the gut microbiome in mice.(19,41)

The gut system contains a large number of opportunistic and pathogenic bacteria that have contributed to the onset, progression, and development of gut diseases. However, phage medicines have been created to target these bacteria. Phage applications for prevention and treatment have resulted in single phages, cocktails, genetically engineered phages, and even a combination with probiotics and antibiotics, among other therapies. Research is being done to create treatments for the most common bacteria linked to infections of the gut system, such as *Salmonella spp*., *Fusobacterium nucleatum*, *Shigella spp.*, *Listeria monocytogenes*, *Ruminococcus gnavus*, *Clostridium difficile*, *Vibrio spp*., *Escherichia coli*, and *Campylobacter spp*.(41)

Many bacteriophage species have been found to have a variety of potential uses, and they are effective therapeutic alternatives to treating strains that are resistant to various drugs, even though the majority are still in the in vitro study, preclinical, and clinical trial stages. These investigations have made it possible to identify and profile phages both singly and in combination with various dose forms and delivery methods. Therapeutic uses of phages and that target gastrointestinal disorders is listed Appendix 1

Conclusions and Future Perspectives

From birth, the gut virome starts to form. The components of a person's gut virome are observably impacted by the mode of delivery. Early feeding practices further alter the gut virome, and the development of the gut flora is significantly influenced by the oligosaccharides in breast milk.

Phages make up most of the gut virome. When taken as a whole, these viruses are crucial for maintaining human health because they regulate the microbiome's composition and mortality. Due to its critical involvement in host metabolism, the gut microbiome's correct functioning is extremely vital. Disturbances in microbial populations are the hallmark of gut dysbiosis, which can affect the host's general health and well-being in addition to the gut. Using FMT and/or FVT to restore core, stable gut microbiota species has demonstrated to have a positive impact in restoring gut health.

The continued evolution of high-throughput sequencing accessibility is allowing our understanding of the gut virome to increase exponentially however further research is required to elucidate the DNA and RNA viromes at various anatomical locations and to connect changes in viral makeup to certain illnesses. According to new research, the virome is frequently influenced by the same factors that affect the human microbiome.

It is crucial to comprehend how bacteriophages affect human health and the gut. These microscopic viral agents are essential for controlling the composition of the gut microbiome, the absorption of

nutrients, the immune system, and pathogen defence. Their capacity to treat illnesses of the gut, such as dysbiosis, and antibiotic resistance presents encouraging opportunities for further therapeutic approaches.

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Appendices

Appendix 1 Bacteriophage Therapy cocktails (Emencheta et al. 2023)

Target Pathogen	Phage Therapy	Disease	Study Type, Model	Reports
Vibrio spp.	Cocktail of five (5) lytic Vibrio phages	Fighting *V. cholerae* infection	In vivo, rabbits	Oral administration of cocktails before infection resulted in prophylactic effects. The phage cocktail significantly reduced bacterial load 6 and 12 h after the challenge.
	Oral phage cocktail therapy	*V. cholerae* infection	In vivo, mice	Phage cocktail (10^8 PFU/mL) given once daily significantly reduced bacterial load.
	Bacteriophage pVp-1	Multiple antibiotic-resistant *V. parahaemolyticus* implicated in gastroenteritis	In vivo, mice	Protection from infection and death 1 h after inoculation with *V. parahaemolyticus*.
	Cocktail of three (3) lytic (virulent) phages—ICP1, ICP2, and ICP3	Cholera pathogenesis/cholera-like diarrhea	In vivo, infant mice and rabbits	Effective at preventing mouse small intestinal colonization. Prophylaxis against the onset of cholera-like diarrhea was achieved after oral administration of the phages up to 24 h before *V. cholera* infestation.
Escherichia coli	Cocktail of three (3) bacteriophages from Siphoviridae and Podoviridae	*E. coli* implicated in gastrointestinal diseases	In vitro	Phage cocktail exhibited broad spectrum and strong lytic activity against *E. coli* isolates.
	Cocktail of lytic phages specific against *E. coli*	Gut pathogenic *E. coli*	In vivo, mice	Suppression of *E. coli* was observed 5–10 d after phage therapy.
	Lytic Myoviridae phage phPE42	Extensively drug-resistant (XDR) *E. coli* implicated in foodborne infections	In vivo, rats	Effective eradication of XDR *E. coli* was observed in animal feces.
	T4-like phages	Childhood diarrhea-associated *E. coli* isolates	In vitro, cultures	T4-like phages combined in a cocktail resulted in increased bacterial lysis.
	Phage-based probiotic dietary supplement consisting of 7 bacteriophage strains	Traveler's diarrhea (TD) caused by *E. coli*, *S. flexneri*, *S. sonnei*, *S. enterica*, *L. monocytogenes*, *S. aureus*	Clinical study, in vivo, humans and mice	Prophylactic effect against TD.
	Specific bacteriophage	Enteropathogenic *E. coli* (EPEC)	In vivo, mice	A single dose of the phage rendered a protective effect on the bacteria throughout the study.
	T4-like coliphages	Acute bacterial diarrhea	Clinical trial, humans	Failure to improve diarrhea condition, possibly due to insufficient phage concentration.
	Virulent bacteriophages targeting prototype of the Adherent Invasive *E. coli* (AIEC) strain LF82	Crohn's disease (CD)	Ex vivo, in vivo, murine and human intestinal samples	Three virulent bacteriophage cocktails were active against the AIEC strain LF82. A single dose of the cocktail reduced colitis symptoms in mice colonized with AIEC.
	Bacteriophage cocktail Ec17B153DK1 vs. the broad-spectrumantibiotic ciprofloxacin	*E. coli* infecting the gut environment	In vitro, simulated small intestine system	The cocktail was effective in reducing *E. coli* in simulated gut conditions. No impact on commensal, non-targeted bacteria.

Target Pathogen	Phage Therapy	Disease	Study Type, Model	Reports
Escherichia coli	Commercial cocktail of *E. coli*-targeting bacteriophages (PreforPro®) containing four phages (*LH01-Myoviridae*, *LL5-Siphoviridae*, *T4D-Myoviridae*, and *LL12-Myoviridae*)	Effect on gut microbiota during GI distress and markers of intestinal and systemic inflammation	Clinical trial, humans	The potential of bacteriophages to selectively reduce target organisms without causing dysbiosis
	Supplemental bacteriophages (PreforPro®)	Enhance the effects of a common probiotic, *B. animalis* subsp. *Lactis* (*B. lactis*) on GI health	Clinical study, humans	Improvements in GI inflammation and colon pain in individuals consuming *B. lactis* with PreforPro®.
	Suppository containing probiotic strains of *Lactobacillus* spp. and bacteriophages specific for pathogenic *E. coli*	Diarrhea	In vivo, calves	Probiotic-phage suppositories reduced the duration of diarrhea in calves. The complete stopping of diarrhea was observed 24–48 h after use.
	Lytic phages (T4, F1, B40-8, and VD13 phages)	Effect on mice gut colonized with human commensal bacteria	In vivo, gnotobiotic mice	Targeted lysis of susceptible gut bacteria. Modulation of non-targeted bacteria through interbacterial interactions.
	Genetically engineered temperate phages	Shiga-toxin (Stx)-producing *E. coli* colonizing the mammalian gut	In vivo, mice	Significant repression of fecal Stx concentrations. Suppression of virulence factors in gut bacteria.
	Phage PDX, a member of the *Myoviridae* family	Diarrheagenic enteroaggregative *E. coli* (EAEC)	In vitro, in vivo, cultures and mice	Bacteriolytic activity of EAEC isolates (EN1E-0007) in vitro and in vivo. No dysbiosis was observed in the anaerobic culture.
	Phage ES17, a Podoviridae phage	Extraintestinal pathogenic *E. coli* (ExPEC) in the intestine	In vivo, mice	Selective elimination of invasive pathobiont species from mucosal surfaces in the intestinal tract.
Clostridium difficile	Six (6) myoviruses and one (1) siphovirus	*C. difficile* infection (CDI)	In vitro, in vivo, hamsters	Specific phage combinations resulted in total lysis of *C. difficile* in vitro. Prevention of resistance. In vivo, the evaluation revealed a reduction in *C. difficile* colonization 36 h post-infection.
	Recombinant bacteriophage	*C. difficile* infection	In vitro, in vivo, cultures and mice	Targeting and killing of *C. difficile*.

Target Pathogen	Phage Therapy	Disease	Study Type, Model	Reports
Salmonella spp.	Phage SE20 (Podoviridae)	*S. enterica* serotype Enteritidis	In vitro, in vivo, mice	Oral administration of a single dose of bacteriophage protected against salmonellosis and treatment of salmonellosis. Animals developed hepatomegaly and splenomegaly as side effects but had no gastrointestinal complications with the phage therapy.
	Bacteriophage cocktail (foodborne outbreak pill (FOP) targeting *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*)	*Salmonella* infection	In vitro	Simulator of the Human Intestinal Microbial Ecosystem (SHIME).
	Phage cocktail	*Salmonella* colonization in experimentally challenged birds	In vivo, birds	Phage treatment effectively reduced Salmonella colonization and enhanced growth performance weight gains in challenged birds.
	Myoviruses and a siphovirus	*Salmonella* infection gastrointestinal enteritis	In vitro, in vivo, swine, birds, cultures	Phage cocktail (STW-77 and SEW-109) had the most lysing efficacy on the swine and bird models. Some phages from the cocktail could lyse resistant strains of the organism.
	Salmonella phages (vB_SenS_KP001, vB_SenS_KP005, and vB_SenS_WP110)	*Salmonella* colonization in the gastrointestinal tract of broilers	In vivo, broilers	The phage cocktail reduced Salmonella colonization in broilers' gastrointestinal tracts from over 70% to 0% 4 d post-treatment.
Fusobacterium nucleatum	Irinotecan-loaded dextran nanoparticles covalently linked to azide-modified phages.	Colorectal cancer (CRC)	In vivo, mice	Phage administration inhibited the growth of *F. nucleatum*. It significantly boosted the effectiveness of first-line chemotherapy treatments for CRC.
	F. nucleatum (*Fn*)-binding M13-phage-loaded silver nanoparticles (AgNPs)	Symbiotic *F. nucleatum* in the gut selectively increases immunosuppressive myeloid-derived suppressor cells (MDSCs), thereby promoting colorectal cancer (CRC) progression.	In vitro, in vivo, mice	Treatment with M13-phage-loaded AgNPs could mop up *F. nucleatum* in the gut, resulting in non-amplification in MDSCs at the tumor sites.
Shigella spp.	*Shigella*-specific bacteriophages: vB_SflS-ISF001, vB_SsoS-ISF002, and a cocktail of both	*S. sonnei* and *S. flexneri* causing human acute gastrointestinal infections	In vitro, cultures	More than 85% of the ESBL-positive and -negative isolates of *S. sonnei* and *S. flexneri* were inhibited by the phage cocktail (vB_SflS-ISF001 and vB_SsoS-ISF002.)

Target Pathogen	Phage Therapy	Disease	Study Type, Model	Reports
Klebsiella pneumoniae	Lytic five-phage combination	Inflammatory bowel disease (IBD)-associated *K. pneumoniae* (Kp) strains	In vivo, mice	Suppression of colitis in mice
	Commercial bacteriophage preparations	*K. pneumoniae* strains isolated from children with functional gastrointestinal disorders (FGIDs)	In vitro, spot test	Phages show negligible lytic activity, indicating the need for a more radical approach to eradicating *K. pneumoniae* in children with FGIDs.
Listeria monocytogenes	Bacteriophage cocktail (Foodborne Outbreak Pill (FOP))	*L. monocytogenes*	In vitro, simulated ilium and colon conditions	Protection against *L. monocytogenes* infecting the human gastrointestinal tract without causing dysbiosis.
Ruminococcus gnavus	Six bacteriophages	Mucin-degrading bacterium *R. gnavus* from the human gut	In vivo, mice	Results show the coexistence of phages with *R. gnavus* in the human gut microbiome.
Campylobacter spp.	Double-stranded phages (Φ 16-izsam and Φ 7-izsam)	*C. jejuni* associated with broilers	In vivo, broilers	Phage administration showed a significant one to two log reduction in *C. jejuni* counts on the cecal content compared with the control group after sacrifice. The lowest colony count was, however, observed with an MOI of 0.1 of Φ 16-izsam.
	Bacteriophages φ4, φ44, φ22, φCj1, φ198, and φ287	*C. jejuni* associated with broilers	In vitro, in vivo, broilers	Demonstrated the susceptibility of a significant number of the multi-resistant *Campylobacter* spp. to the phage isolates, which had a lytic spectrum of 6, 4, 4, 3, 8, and 7, respectively.
General	Chitosan-encapsulated bacteriophage cocktail	*S. enterica*, *S. flexneri*, and *E. coli* gastrointestinal infections	In vivo, rats	Reduction in positive cultures from stools of the group receiving the chitosan-encapsulated bacteriophage cocktail was observed after two days.

Summary of studies assessing the enteric virome in patients with Inflammatory Bowel Disease (Mukhopadhya et al. 2019)

Study	Year	Patient cohort	Age group	Sample source	Number of patients	Number of controls	Method	Key findings
Lepage *et al.*	2008	CD	Adult	Colonic biopsies	19	14	Epifluorescence microscopy, transmission electron microscopy	↑Bacteriophages detected in the mucosa from CD patients than from healthy individuals
Wagner *et al.*	2013	CD	Paediatric	Ileal and colonic biopsies, gut wash samples	6, 3	8	Viral metagenome – 454 pyrosequencing Roche GS-FLX Titanium	Differences in bacteriophage composition between CD patients and control individuals
Pérez-Brocal *et al.*	2013	CD	Adult	Faeces, ileum tissue	11, 1	6	Viral metagenome – 454 pyrosequencing Roche GS- FLX titanium plus	↓ Diversity of viral and bacterial communities in CD samples compared with the control group ↑ Variability between the CD samples in both virome and microbiome
Wang *et al.*	2015	CD + UC	Adult	Colonic tissue (biopsy/ surgery)	10	5	Viral metagenome – Illumina HiSeq 2000 sequencing platform	↑ Viral sequences in CD Difference in abundance and diversity within the virome between CD and control group
Norman *et al.*	2015	CD + UC	Adult	Faeces	UK Cohort (UC 21, CD 14) Chicago cohort (UC 17, CD 8) LA cohort (UC 22, CD 1) Boston cohort (UC 15, CD 20)	UK Cohort (HC 22) Chicago cohort (HC 24) LA cohort (HC 0) Boston cohort (HC 10)	Viral metagenome – Roche 454 (initial study) and Illumina MiSeq platform (in-depth analysis)	↑ Viral richness and Caudovirales expansion in CD and UC ↓ Decreased bacterial richness and diversity in UC and CD Inverse correlation of Caudovirales with prevalent bacterial taxa in CD

UC, ulcerative colitis; CD, Crohn's disease; HC, Healthy controls.

Extended disease examples and viral population alterations in human disorders. (Liang & Bushman, 2021)

Human disease	Sample	Major virome alteration
Severe acute malnutrition	Faeces	Reduced viral diversity
Crohn's disease and ulcerative colitis	Faeces	Increased *Caudovirales* richness
Crohn's disease	Faeces and biopsies	Moderate alterations
AIDS	Faeces	Increased enteric adenoviruses
Type 1 diabetes	Faeces	Reduced viral diversity
Hypertension	Faeces	*Erwinia* phage ΦEaH2 and *Lactococcus* phage 1706 may be associated with hypertension
Type 2 diabetes	Faeces	Increased putative phage scaffolds
DOCK8 deficiency	Skin swabs	Increased skin virome, especially human papillomavirus
Colorectal cancer	Faeces	Increased viral diversity
Crohn's disease and ulcerative colitis	Faeces	Increased *Caudovirales* abundance
Type 1 diabetes during pregnancy	Faeces	Increased picobirnaviruses and tobamoviruses
Bacterial vaginosis	Vaginal swabs	Viral population structures correlated with bacterial vaginosis
Early-diagnosed Crohn's disease and ulcerative colitis	Gut biopsies	Increased *Hepadnaviridae* and *Hepeviridae*; reduced *Polydnaviridae*, *Tymoviridae* and *Virgaviridae*
Coeliac disease autoimmunity	Faeces	Increased enteroviruses
Crohn's disease	Faeces	The virulent phages are replaced with temperate phages
Ulcerative colitis	Gut biopsies	Increased *Caudovirales*, phage and bacteria virulence functions, and loss of viral–bacterial correlations
HIV viraemia	Seminal fluid	Increased human cytomegalovirus
Very early-onset inflammatory bowel disease	Faeces	Increased ratio of *Caudovirales* to *Microviridae*
Haematopoietic stem cell transplantation	Faeces	Increased picobirnaviruses