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Review article

The emerging role of the gut virome in necrotizing enterocolitis

Cong Yi, Jia Chen, Xiang She *

Department of Pediatrics, Mianyang Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Mianyang, 621000, China

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ABSTRACT

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in neonates, particularly preterm infants. Many factors can lead to NEC, but microbial dysbiosis is one of the most important risk factors that can induce this disease. Given the major role of the gut virome in shaping bacterial homeostasis, virome research is a fledgling but rapidly evolving area in the field of microbiome that is increasingly connected to human diseases, including NEC. This review provides an overview of the development of the gut virome in newborns, discusses its emerging role in NEC, and explores promising therapeutic applications, including phage therapy and fecal virome transplantation.

1. Introduction

Necrotizing enterocolitis (NEC), a gastrointestinal emergency that primarily affects premature and very-low-birthweight newborns, poses high morbidity and mortality risks in neonates, with potential long-term consequences into adulthood [1,2]. Increased survival of premature infants has heightened NEC incidence. While the precise pathophysiology of NEC remains poorly understood, microbial dysbiosis, particularly of the gut microbiome, is regarded as a significant contributor to its initiation and development. Using deep sequencing techniques, Breitbart et al. [3] first revealed high viral diversity in a healthy adult feces. The gut virome, as the main member of gut microecology, plays an important role in health and disease. The expansion of *Caudovirales* and the reduction of *Microviridae* are the most consistently observed change of gut virome in IBD [4–6]. Although gut bacteria are likely involved in NEC pathogenesis, recent studies suggest a connection between the gut virome and NEC etiology [7,8]. This review explores the development of the gut virome in newborns, summarizes recent evidence on its role in NEC, and discusses its promising therapeutic applications.

2. Development of the gut virome in newborns

The human gut microbiome is a complex system comprising bacteria, archaea, fungi, and viruses. The human virome is equally complex, containing approximately 10^{13} virus-like particles (VLPs) per adult. The gut is the most densely virally colonized region in the body, with at least 10^{9} VLPs per gram of human feces [9]. The gut virome mainly comprises bacteriophages (phages) that infect bacteria and eukaryotic viruses that infect human cells, of which phages account for approximately 90 %. In contrast to adults, the neonatal gut virome develops in a unique and stepwise process, with VLPs generally absent in healthy meconium or amniotic fluids but rapidly colonizing the gut afterward [10–12]. The number of VLPs increases to 10^{8} VLPs per gram of intestinal content within the first

* Corresponding author.
E-mail address: xiangshe6784@126.com (X. She).

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week of life and then to 10^9 VLPs after one month, similar to adult levels [11,13].

The abundance and diversity of eukaryotic viruses in the gut are scarce in early life but gradually increase. Astroviruses are most commonly found in preterm infants [14,15]. Phages are generally classified into lytic or temperate. Lytic phages infect, replicate, and lyse bacterial cells, releasing new phage particles. Conversely, temperate phages integrate their DNA into the host genome, entering a quiescent state until stimulation triggers the lytic cycle. Early neonatal gut viral colonization involves prophages integrated in pioneering bacteria, providing the first wave of viral particles [13]. The most abundant phage order, *Caudovirales*, primarily includes the families *Myoviridae, Siphoviridae,* and *Podoviridae*, which influence the early intestinal microbiota colonization of *Firmicutes, Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* [15–17]. Recently, the International Committee on Taxonomy of Viruses abolished the order *Caudovirales* and the families *Myoviridae*, *Siphoviridae*, and *Podoviridae* [18]. Subsequently, the gut virome undergoes a transition from *Caudovirales*-to *Microviridae*-dominated communities in the first two years of life [13,15]. Phages and bacteria have a dynamic interaction and mutually affect one another. Phage richness and diversity is higher during the first week of life, with an age-dependent negative correlation between phages and bacterial richness suggesting prey-predator dynamics. Both phages and bacteria reach a stable, adult-like composition around 2–3 years of age [15].

3. Factors shaping the neonatal gut virome composition

The initial gut virome stems from induced prophages of pioneering gut bacteria, akin to early life bacterial colonization, and is affected by multiple factors such as birth mode, feeding method, and antibiotic use. Vaginally born infants have greater viral diversity than those born via cesarean section, with *Caudoviricetes*, *Microviridae*, and *Anelloviridae* being the predominant viruses [9,19]. Infant feeding variations also impact the gut virome. Maternal milk, vital in early virome transmission and establishment beginning within hours of birth, shares high virome similarity with infant stool, including the presence of bifidobacteria bacteriophages, indicating potential vertical transmission of the maternal virome to the gut of infants through breast milk [20,21]. Breast feeding fosters beneficial bacteria and associated phages, inhibiting eukaryotic viral colonization, while fecal eukaryotic viral loads are higher in formula-fed infants [13,22]. Nonetheless, certain viruses that have infected the mother can be passed on through breastfeeding (e.g., cytomegalovirus, human immunodeficiency virus, and hepatitis B virus) [23,24]. Hospitalized preterm infants are vulnerable to infection and often require antibiotics, which significantly decrease their intestinal microbiota diversity [25]. Initially, the gut virome diversity increases sharply with the release of new viral particles but eventually decreases owing to host loss.

4. Gut virome role in NEC pathogenesis

4.1. Phages and NEC

Phages are a group of prokaryotic viruses that infect bacterial cells and are among the least understood biological entities. Structurally, the majority of phages consist of a viral genetic material enclosed by a protein shell (capsid), while certain phages may also possess an external lipid membrane or a lipoprotein envelope [26,27]. Genome sizes vary greatly (~3.5–~540 kb) and consist of single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), or double-stranded RNA (dsRNA) [27]. Morphologically, phages are tailed, polyhedral, pleomorphic, or filamentous [28].

Phages affect the composition and function of bacterial communities and help maintain intestinal homeostasis by increasing functional diversity within bacterial or phage communities [29]. Phages are linked to beneficial immune maturation via TLR-9 [30]. However, gut virome dysbiosis can reduce bacterial richness and diversity, leading to gut microbiota dysbiosis. Recent studies link changes in the gut virome with various diseases, including diabetes, colon cancer, IBD, and obesity [4,31,32]. A recent study identified a tendency towards decreased viral beta diversity in the 10-day period prior to the onset of NEC, characterized by specific viral signatures and viral-bacterial interactions [8]. Nevertheless, the study sample size was small and the causal relationship between changes in viral community composition and NEC requires further investigation through extensive translational animal experiments and prospective longitudinal human trials.

4.2. Eukaryotic viruses and NEC

Eukaryotic viruses constitute only a small fraction of the healthy human gut virome and include ssDNA viruses (*Anelloviridae* and *Circoviridae*), dsDNA viruses (*Adenoviridae*, *Herpesviridae*, *Papillomaviridae*, and *Polyomaviridae*), ssRNA viruses (*Virgaviridae*), and dsRNA viruses (*Reoviridae*) [33–35]. Some eukaryotic viruses may cause infection or remain latent, while others engage in benign colonization and are unrelated to specific diseases [9]. Each dsDNA virus and some RNA viruses are associated with clinically infectious diseases, whereas ssDNA viruses are frequently not [36]. Some eukaryotic viruses linked to human diseases have received greater research attention in comparison to other members of the gut virome.

Several viruses that cause gastroenteritis are thought to be associated with NEC development. Eukaryotic viruses like echovirus, coronavirus, norovirus, and rotavirus play key etiological roles in NEC outbreaks [37–40]. Other viruses such as human astrovirus, adenovirus, torovirus, and cytomegalovirus have been detected in sporadic NEC [14,41–44]. A meta-analysis of 24 studies with 4356 infants demonstrated that viral infection is linked to increased risk of NEC in newborns, especially infection with rotavirus, cytomegalovirus, norovirus, and astrovirus [7]. However, different viruses have been found in different studies, and even no specific viral pathogens have been detected in some studies [45,46]. Thus, the current findings appear inconsistent.

The pathogenic roles of specific eukaryotic viruses have been extensively studied. Ginze et al. [47] demonstrated that the viral

dsRNA analog polyinosinic:polycytidylic acid leads to increased intestinal tissue damage in the absence of lipopolysaccharide in neonatal NEC mice. Notably, recent studies have shown that eukaryotic viruses can exert beneficial functions, akin to those of commensal bacteria, by influencing host immunity. Enteric viruses alleviate intestinal inflammation by Toll-like receptor 3 and Toll-like receptor 7-mediated interferon-β production [48]. Murine norovirus (MNV) is a common enteric RNA virus that reverses intestinal abnormalities in germ-free or antibiotic-treated mice [49]. NS1/2, the viral protein from MNV, evokes protective responses from the host depending on the type I interferon response and interleukin (IL)-22 expression, which contribute to epithelial proliferation and protection from intestinal injury [50]. Hu et al. [51] also found that MNV plays a protective role in intestinal injury in neonatal NEC mice by IL-22.

5. Virome-based therapies in NEC

5.1. Phage therapy

Phages are host-specific bacterial viruses first used in the early 1900s to treat bacterial infections [52]. The discovery and widespread availability of antibiotics has led to a decline in phage research. However, the emergence and rapid spread of antibiotic-resistant pathogens have generated renewed interest in phage therapy [53]. Currently, single-phage therapy faces limitations in clinical use owing to a narrow phage lysis spectrum and the emergence of phage-resistant bacteria [54,55]. While the specific causative pathogen of NEC remains unknown, numerous studies have revealed the potential role of bacteria in NEC pathogenesis. Therefore, phage cocktail therapy may be a viable option.

Phage cocktails consist of a mixture of different phages that can broaden the range of susceptible hosts and minimize the production of phage-resistant bacteria. In 2009, Rhoads et al. [56] conducted a phase 1 trial to confirm the safety of a phage cocktail targeting *Pseudomonas aeruginosa*, *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* to treat venous leg ulcers in humans. The therapeutic research and clinical application of phage have since gradually emerged in Europe and America. In gastroenterology, phage therapy research has largely focused on infectious diseases. Adherent invasive *E. coli* (AIEC) is implicated in the pathogenesis of Crohn's disease (CD) [57]; a phage cocktail targeting AIEC strains effectively reduces AIEC intestinal colonization and intestinal inflammation in mice, demonstrating promise as an alternative treatment for CD patients, as evidenced in homogenates from ileal biopsies [58]. A seven lytic phage cocktail targeting AIEC (EcoActive) showed efficacy against 95 % of the 210 clinical AIEC strains *in vitro* and alleviated inflammatory symptoms in a mouse LF82 strain-induced colitis model [59]. Meanwhile, a phase 1/2a randomized, double-blind, placebo-controlled trial (NCT03808103) evaluating the safety and efficacy of EcoActive on AIEC in patients with inactive CD is ongoing. Although *in vivo* and *in vitro* studies on phage therapy show encouraging results for clinical applications, further studies are required to establish its safety, efficacy, and universal acceptance in modern clinical practice. Unfortunately, no studies on phage therapy for NEC have been conducted.

5.2. Fecal virome transplantation

Fecal microbiota transplantation (FMT) is a promising treatment for dysbiosis-related diseases, including *Clostridioides difficile* infection, IBD, obesity, and diabetes [60–65]. Gut microbiota dysbiosis, marked by reduced diversity of bacterial species, elevated abundance of *Proteobacteria*, and reduced abundance of *Firmicutes* and *Bacteroidetes*, is evident in patients with NEC [66–68]. FMT in preclinical studies prevents NEC by improving bacterial colonization and barrier function and decreasing intestinal injury, oxidative stress, and inflammation [67,69–71]. However, safety and feasibility concerns surround FMT in NEC. Liu et al. [69] found that oral FMT further aggravated inflammation in the terminal ileum and did not ameliorate the histological damage caused by experimental NEC. Brunse et al. [72] also demonstrated that oral FMT increased sepsis-related mortality due to the exposure of gut to potential pathogens in the stool of donor.

Faecal virome transplantation (FVT) is a refinement to FMT that transplants the sterile donor fecal filtrate containing phages but no intact bacterial cells, thereby reducing the risk of FMT-associated bacterial infections. Recent studies suggest phages play an essential role in the success of FMT treatment by control of disease progression and restoration of intestinal microbiota balance [73–77]. Draper et al. [74] demonstrated the co-transfer and enduring engraftment of phages during FMT, which is potentially crucial for stabilizing the post-FMT microbiome. A recent study reported that oral FVT administration completely prevented NEC in a piglet model, whereas FMT failed to prevent NEC, demonstrating its higher safety and efficacy without recognizable side effects [78]. While the study cannot rule out the potential influence of microbial metabolites, secreted proteins, and other fecal filtrate substances, it highlighted the significance of exploring the involvement of the virome in FMT. However, due to the presence of potentially pathogenic viruses in the intestines of healthy donors, such as herpes virus, hepatitis virus, bocavirus, enterovirus, rotavirus, and sapovirus [34], FVT poses a potential risk to some neonates, demanding the comprehensive screening of the donor fecal virome and continuous monitoring of the recipient gut virome to avoid health complications. Given the limited studies on the role of FVT in treatment of NEC, further research is necessary to investigate the FVT-transferred viral community, its interactions with host and gut microbiota, and its short- and long-term effects.

6. Conclusions

The gut virome is an emerging research area that faces challenges due to viral metagenomics methodological limitations, leaving numerous viruses unclassified as "viral dark matter" [79]. Fortunately, the infant gut viral dark matter has been largely resolved and

made available online for the infant gut viromics research community to utilise [80]. However, there is limited knowledge regarding the composition, changes, and functions of the gut virome in NEC. Further research is required to improve our comprehension and develop innovative clinical therapies.

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Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Cong Yi: Writing – original draft, Project administration, Data curation, Conceptualization. **Jia Chen:** Writing – review & editing, Project administration, Data curation. **Xiang She:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J. Neu, W.A. Walker, Necrotizing enterocolitis, N. Engl. J. Med. 364 (3) (2011) 255–264.
[2] D.J. Hackam, C.P. Sodhi, Bench to bedside - new insights into the pathogenesis of necrotizing enterocolitis, Nat. Rev. Gastroenterol. Hepatol. 19 (7) (2022) 468–479.
[3] M. Breitbart, I. Hewson, B. Felts, et al., Metagenomic analyses of an uncultured viral community from human feces, J. Bacteriol. 185 (20) (2003) 6220–6223.
[4] G. Liang, A.G. Cobián-Güemes, L. Albenberg, F. Bushman, The gut virome in inflammatory bowel diseases, Curr Opin Virol 51 (2021) 190–198.
[5] J.M. Norman, S.A. Handley, M.T. Baldridge, et al., Disease-specific alterations in the enteric virome in inflammatory bowel disease, Cell 160 (3) (2015) 447–460.
[6] A.G. Clooney, T.D.S. Sutton, A.N. Shkoporov, et al., Whole-virome analysis sheds light on viral dark matter in inflammatory bowel disease, Cell Host Microbe 26 (6) (2019) 764–778.e5.
[7] S. Mani, S. Hazra, J. Hagan, A. Sisson, J. Nair, M. Pammi, Viral infections and neonatal necrotizing enterocolitis: a meta-analysis, Pediatrics 152 (1) (2023) e2022060876.
[8] E.A. Kaelin, C. Rodriguez, C. Hall-Moore, et al., Longitudinal gut virome analysis identifies specific viral signatures that precede necrotizing enterocolitis onset in preterm infants, Nat Microbiol 7 (5) (2022) 653–662.
[9] G. Liang, F.D. Bushman, The human virome: assembly, composition and host interactions, Nat. Rev. Microbiol. 19 (8) (2021) 514–527.
[10] L. Brazier, E. Elguero, C.K. Koumavor, et al., Evolution in fecal bacterial/viral composition in infants of two central African countries (Gabon and Republic of the Congo) during their first month of life, PLoS One 12 (10) (2017) e0185569.
[11] M. Breitbart, M. Haynes, S. Kelley, et al., Viral diversity and dynamics in an infant gut, Res. Microbiol. 159 (5) (2008) 367–373.
[12] E.S. Lim, C. Rodriguez, L.R. Holtz, Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community [published correction appears in Microbiome. 2019 Feb 12;7(1):22], Microbiome 6 (1) (2018) 87.
[13] G. Liang, C. Zhao, H. Zhang, et al., The stepwise assembly of the neonatal virome is modulated by breastfeeding, Nature 581 (7809) (2020) 470–474.
[14] C. Cheng, Y. He, S. Xiao, Q. Ai, J. Yu, The association between enteric viruses and necrotizing enterocolitis, Eur. J. Pediatr. 180 (1) (2021) 225–232.
[15] E.S. Lim, Y. Zhou, G. Zhao, et al., Early life dynamics of the human gut virome and bacterial microbiome in infants, Nat. Med. 21 (10) (2015) 1228–1234.
[16] A. Reyes, L.V. Blanton, S. Cao, et al., Gut DNA viromes of Malawian twins discordant for severe acute malnutrition, Proc. Natl. Acad. Sci. U.S.A. 112 (38) (2015) 11941–11946.
[17] A. Reyes, M. Haynes, N. Hanson, et al., Viruses in the faecal microbiota of monozygotic twins and their mothers, Nature 466 (7304) (2010) 334–338.
[18] D. Turner, A.N. Shkoporov, C. Lood, et al., Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee, Arch. Virol. 168 (2) (2023) 74.
[19] A. McCann, F.J. Ryan, S.R. Stockdale, et al., Viromes of one year old infants reveal the impact of birth mode on microbiome diversity, PeerJ 6 (2018) e4694.
[20] P.S. Pannaraj, M. Ly, C. Cerini, et al., Shared and distinct features of human milk and infant stool viromes, Front. Microbiol. 9 (2018) 1162.
[21] S. Duranti, G.A. Lugli, L. Mancabelli, et al., Maternal inheritance of bifidobacterial communities and bifidophages in infants through vertical transmission, Microbiome 5 (1) (2017) 66.
[22] F. Bushman, G. Liang, Assembly of the virome in newborn human infants, Curr Opin Virol 48 (2021) 17–22.
[23] J.S. Lawson, J. Heads, W.K. Glenn, N.J. Whitaker, Breastfeeding, breast milk and viruses, BMC Wom. Health 7 (2007) 17.
[24] L.M. Kemeter, A. Birzer, S. Heym, A.K. Thoma-Kress, Milk transmission of mammalian retroviruses, Microorganisms 11 (7) (2023) 1777.
[25] Z.H. Zou, D. Liu, H.D. Li, et al., Prenatal and postnatal antibiotic exposure influences the gut microbiota of preterm infants in neonatal intensive care units, Ann. Clin. Microbiol. Antimicrob. 17 (1) (2018) 9.
[26] L.K. Harada, E.C. Silva, W.F. Campos, et al., Biotechnological applications of bacteriophages: state of the art, Microbiol. Res. 212–213 (2018) 38–58.
[27] L.M. Kasman, L.D. Porter, Bacteriophages. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC., 2022.
[28] G.P. Salmond, P.C. Fineran, A century of the phage: past, present and future, Nat. Rev. Microbiol. 13 (12) (2015) 777–786.
[29] B.B. Hsu, T.E. Gibson, V. Yeliseyev, et al., Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model, Cell Host Microbe 25 (6) (2019) 803–814.e5.
[30] Rodríguez C. Leal, S.A. Shah, M.A. Rasmussen, et al., The infant gut virome is associated with preschool asthma risk independently of bacteria, Nat. Med. 30 (1) (2024) 138–148.
[31] L. Massimino, S. Lovisa, L. Antonio Lamparelli, S. Danese, F. Ungaro, Gut eukaryotic virome in colorectal carcinogenesis: is that a trigger? Comput. Struct. Biotechnol. J. 19 (2020) 16–28.
[32] G. Tarris, A. de Rougemont, M. Charkaoui, C. Michiels, L. Martin, G. Belliot, Enteric viruses and inflammatory bowel disease, Viruses 13 (1) (2021) 104.

[33] B. Taboada, P. Morán, A. Serrano-Vázquez, et al., The gut virome of healthy children during the first year of life is diverse and dynamic, PLoS One 16 (4) (2021) e0240958.
[34] I. Mukhopadhya, J.P. Segal, S.R. Carding, A.L. Hart, G.L. Hold, The gut virome: the 'missing link' between gut bacteria and host immunity? Therap Adv Gastroenterol 12 (2019) 1756284819836620.
[35] Y. Li, S.A. Handley, M.T. Baldridge, The dark side of the gut: virome-host interactions in intestinal homeostasis and disease, J. Exp. Med. 218 (5) (2021) e20201044.
[36] V. Fulci, L. Stronati, S. Cucchiara, I. Laudadio, C. Carissimi, Emerging roles of gut virome in pediatric diseases, Int. J. Mol. Sci. 22 (8) (2021) 4127.
[37] S.A. Coggins, J.L. Wynn, J.H. Weitkamp, Infectious causes of necrotizing enterocolitis, Clin. Perinatol. 42 (1) (2015) 133–154, ix.
[38] R.L. Stuart, K. Tan, J.E. Mahar, et al., An outbreak of necrotizing enterocolitis associated with norovirus genotype GII.3, Pediatr. Infect. Dis. J. 29 (7) (2010) 644–647.
[39] E. Birenbaum, R. Handsher, J. Kuint, et al., Echovirus type 22 outbreak associated with gastro-intestinal disease in a neonatal intensive care unit, Am. J. Perinatol. 14 (8) (1997) 469–473.
[40] H.A. Rotbart, W.L. Nelson, M.P. Glode, et al., Neonatal rotavirus-associated necrotizing enterocolitis: case control study and prospective surveillance during an outbreak, J. Pediatr. 112 (1) (1988) 87–93.
[41] M. Shimamura, Is there a link between viruses and necrotising enterocolitis? Acta Paediatr. 105 (1) (2016) 15–16.
[42] S. Bagci, A.M. Eis-Hübinger, A.R. Franz, et al., Detection of astrovirus in premature infants with necrotizing enterocolitis, Pediatr. Infect. Dis. J. 27 (4) (2008) 347–350.
[43] R.M. Patel, N. Shenvi, A. Knezevic, et al., Observational study of cytomegalovirus from breast milk and necrotising enterocolitis, Arch. Dis. Child. Fetal Neonatal Ed. 105 (3) (2019) 259–265.
[44] S. Panesso-Gómez, M. Shimamura, M. Conces, et al., Detection of cytomegalovirus in intestinal tissue of infants with necrotizing enterocolitis or spontaneous intestinal perforation, J. Pediatr. 214 (2019) 34–40.
[45] T. Skeath, C. Stewart, S. Waugh, N. Embleton, S. Cummings, J. Berrington, Cytomegalovirus and other common enteric viruses are not commonly associated with NEC, Acta Paediatr. 105 (1) (2016) 50–52.
[46] T. Ullrich, Y.W. Tang, H. Correa, et al., Absence of gastrointestinal pathogens in ileum tissue resected for necrotizing enterocolitis, Pediatr. Infect. Dis. J. 31 (4) (2012) 413–414.
[47] M. Ginzel, Y. Yu, C. Klemann, et al., The viral dsRNA analogue poly (I:C) induces necrotizing enterocolitis in neonatal mice, Pediatr. Res. 79 (4) (2016) 596–602.
[48] J.Y. Yang, M.S. Kim, E. Kim, et al., Enteric viruses ameliorate gut inflammation via toll-like receptor 3 and toll-like receptor 7-mediated interferon-β production, Immunity 44 (4) (2016) 889–900.
[49] E. Kernbauer, Y. Ding, K. Cadwell, An enteric virus can replace the beneficial function of commensal bacteria, Nature 516 (7529) (2014) 94–98.
[50] J.A. Neil, Y. Matsuzawa-Ishimoto, E. Kernbauer-Hölzl, et al., IFN-I and IL-22 mediate protective effects of intestinal viral infection, Nat Microbiol 4 (10) (2019) 1737–1749.
[51] H. Kun, Y. Jialin, A. Qing, et al., Effect of murine norovirus on the intestinal injury in neonatal mouse with necrotizing enterocolitis, Acta Acad Med Mil Tert 40 (10) (2018) 898–903.
[52] V.V. Morozova, V.V. Vlassov, N.V. Tikunova, Applications of bacteriophages in the treatment of localized infections in humans, Front. Microbiol. 9 (2018) 1696.
[53] K.E. Kortright, B.K. Chan, J.L. Koff, P.E. Turner, Phage therapy: a renewed approach to combat antibiotic-resistant bacteria, Cell Host Microbe 25 (2) (2019) 219–232.
[54] J.Y. Nale, J. Spencer, K.R. Hargreaves, et al., Bacteriophage combinations significantly reduce Clostridium difficile growth in vitro and proliferation in vivo, Antimicrob. Agents Chemother. 60 (2) (2015) 968–981.
[55] H. Raeisi, M. Noori, M. Azimirad, et al., Emerging applications of phage therapy and fecal virome transplantation for treatment of Clostridioides difficile infection: challenges and perspectives, Gut Pathog. 15 (1) (2023) 21.
[56] D.D. Rhoads, R.D. Wolcott, M.A. Kuskowski, B.M. Wolcott, L.S. Ward, A. Sulakvelidze, Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial, J. Wound Care 18 (6) (2009) 237–243.
[57] C. Palmela, C. Chevarin, Z. Xu, et al., Adherent-invasive *Escherichia coli* in inflammatory bowel disease, Gut 67 (3) (2018) 574–587.
[58] M. Galtier, L. De Sordi, A. Sivignon, et al., Bacteriophages targeting adherent invasive Escherichia coli strains as a promising new treatment for Crohn's disease, J Crohns Colitis 11 (7) (2017) 840–847.
[59] M. Titécat, C. Rousseaux, C. Dubuquoy, et al., Safety and efficacy of an AIEC-targeted bacteriophage cocktail in a mice colitis model, J Crohns Colitis 16 (10) (2022) 1617–1627.
[60] M. Hsu, K.M. Tun, K. Batra, L. Haque, T. Vongsavath, A.S. Hong, Safety and efficacy of fecal microbiota transplantation in treatment of inflammatory bowel disease in the pediatric population: a systematic review and meta-analysis, Microorganisms 11 (5) (2023) 1272.
[61] A. Khoruts, C. Staley, M.J. Sadowsky, Faecal microbiota transplantation for Clostridioides difficile: mechanisms and pharmacology, Nat. Rev. Gastroenterol. Hepatol. 18 (1) (2021) 67–80.
[62] L. Zhu, J. Fu, X. Xiao, et al., Faecal microbiota transplantation-mediated jejunal microbiota changes halt high-fat diet-induced obesity in mice via retarding intestinal fat absorption, Microb. Biotechnol. 15 (1) (2022) 337–352.
[63] Y. Wang, Z. Yang, H. Tang, et al., Faecal microbiota transplantation is better than probiotics for tissue regeneration of type 2 diabetes mellitus injuries in mice, Arch. Physiol. Biochem. (2022) 1–9.
[64] S.C. Ng, Z. Xu, J.W.Y. Mak, et al., Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial, Gut 71 (4) (2022) 716–723.
[65] N.Z. Minkoff, S. Aslam, M. Medina, et al., Fecal microbiota transplantation for the treatment of recurrent Clostridioides difficile (Clostridium difficile), Cochrane Database Syst. Rev. 4 (4) (2023) CD013871.
[66] M. Pammi, J. Cope, P.I. Tarr, et al., Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis, Microbiome 5 (1) (2017) 31.
[67] Y. He, W. Du, S. Xiao, et al., Colonization of fecal microbiota from patients with neonatal necrotizing enterocolitis exacerbates intestinal injury in germfree mice subjected to necrotizing enterocolitis-induction protocol via alterations in butyrate and regulatory T cells, J. Transl. Med. 19 (1) (2021) 510.
[68] B.B. Warner, E. Deych, Y. Zhou, et al., Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study, Lancet 387 (10031) (2016) 1928–1936.
[69] J. Liu, H. Miyake, H. Zhu, et al., Fecal microbiota transplantation by enema reduces intestinal injury in experimental necrotizing enterocolitis, J. Pediatr. Surg. 55 (6) (2020) 1094–1098.
[70] C. Prado, M. Michels, P. Ávila, H. Burger, M.V.M. Milioli, F. Dal-Pizzol, The protective effects of fecal microbiota transplantation in an experimental model of necrotizing enterocolitis, J. Pediatr. Surg. 54 (8) (2019) 1578–1583.
[71] C. Prado, M.R. Abatti, M. Michels, et al., Comparative effects of fresh and sterile fecal microbiota transplantation in an experimental animal model of necrotizing enterocolitis, J. Pediatr. Surg. 57 (9) (2022) 183–191.
[72] A. Brunse, L. Martin, T.S. Rasmussen, et al., Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs, ISME J. 13 (3) (2019) 720–733.
[73] T. Zuo, S.H. Wong, K. Lam, et al., Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome, Gut 67 (4) (2018) 634–643.
[74] L.A. Draper, F.J. Ryan, M.K. Smith, et al., Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation, Microbiome 6 (1) (2018) 220.
[75] L.A. Draper, F.J. Ryan, M. Dalmasso, et al., Autochthonous faecal viral transfer (FVT) impacts the murine microbiome after antibiotic perturbation, BMC Biol. 18 (1) (2020) 173.

[76] S.J. Ott, G.H. Waetzig, A. Rehman, et al., Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection, Gastroenterology 152 (4) (2017) 799–811.e7.
[77] T.S. Rasmussen, C.M.J. Mentzel, W. Kot, et al., Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model, Gut 69 (12) (2020) 2122–2130.
[78] A. Brunse, L. Deng, X. Pan, et al., Fecal filtrate transplantation protects against necrotizing enterocolitis, ISME J. 16 (3) (2022) 686–694.
[79] S.R. Krishnamurthy, D. Wang, Origins and challenges of viral dark matter, Virus Res. 239 (2017) 136–142.
[80] S.A. Shah, L. Deng, J. Thorsen, et al., Expanding known viral diversity in the healthy infant gut, Nat Microbiol 8 (5) (2023) 986–998.