REVIEW

Modulation of gut microbiota protects against viral respiratory tract infections: a systematic review of animal and clinical studies

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Abstract

Background Earlier studies suggest that probiotics have protective effects in the prevention of respiratory tract infections (RTIs). Whether such benefits apply to RTIs of viral origin and mechanisms supporting the effect remain unclear.

Aim To determine the role of gut microbiota modulation on clinical and laboratory outcomes of viral RTIs.

Methods We conducted a systematic review of articles published in Embase and MEDLINE through 20 April 2020 to identify studies reporting the effect of gut microbiota modulation on viral RTIs in clinical studies and animal models. The incidence of viral RTIs, clinical manifestations, viral load and immunological outcomes was evaluated.

Results We included 58 studies (9 randomized controlled trials; 49 animal studies). Six of eight clinical trials consisting of 726 patients showed that probiotics administration was associated with a reduced risk of viral RTIs. Most commonly used probiotics were *Lactobacillus* followed by *Bifidobacterium* and *Lactococcus*. In animal models, treatment with probiotics before viral challenge had beneficial effects against influenza virus infection by improving infection-induced survival (20/22 studies), mitigating symptoms (21/21 studies) and decreasing viral load (23/25 studies). Probiotics and commensal gut microbiota exerted their beneficial effects through strengthening host immunity.

Conclusion Modulation of gut microbiota represents a promising approach against viral RTIs via host innate and adaptive immunity regulation. Further research should focus on next generation probiotics specific to viral types in prevention and treatment of emerging viral RTIs.

Abbreviations

BALF	Bronchoalveolar lavage fluid
COVID-19	Coronavirus disease 2019
DCs	Dendritic cells
FMT	Fecal microbiota transplantation
HSCT	Hematopoietic stem cell transplantation
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
MERS-CoV	Middle East respiratory syndrome coronavirus
NK	Natural killer
RCTs	Randomized controlled trials

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RR	Rate ratio
RSV	Respiratory syncytial virus
RTIs	Respiratory tract infections
SARS-CoV	Severe acute respiratory syndrome coronavirus
SCFAs	Short-chain fatty acids
Th1	T helper type 1
Th2	T helper type 2
TNF	Tumor necrosis factor

Introduction

Viral respiratory tract infections (RTIs) are major global public health challenges because they are the most common causes of infectious diseases resulting in work and productivity loss [1, 2]. Effective antiviral drugs and vaccinations are lacking for non-influenza respiratory viruses [1, 3]. The outbreak of novel viruses causing high mortality, including severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003, influenza A (H5N1) in 2005, Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, and influenza A (H7N9) in 2013 [4] and more recently, SARS-CoV-2 leading to novel coronavirus disease 2019 (COVID-19) has resulted in catastrophic outcomes. As of 30 Jan 2021, COVID-19 has infected over 102.6 million people and led to more than 2.2 million deaths worldwide. Given the challenges on both of the prevention and treatment of viral RTIs, it is important to identify effective and safe measures to protect against emerging infectious diseases.

Gut microbiota plays pivotal roles in establishing intestinal mucosal barrier function [5], shaping nutrient absorption and metabolism [6], and modulating host immunity [7]. Maintenance of microbiota homeostasis has been implicated to be crucial for creating colonization resistance to foreign pathogens [8]. Particularly, microbiota-mediated triggering, calibrating and functioning of both innate and adaptive immunity assist in facilitating protection against exogenous viruses [9, 10]. In contrast, a dysregulated microbiota composition which loses elasticity and diversity is more likely to provoke impaired immune responses [11].

Previous systematic reviews have identified the protective effects of oral probiotics and prebiotics in the prevention of RTIs [12–16]. However, very few studies included in these systematic reviews included virological tests whereas the majority studies defined RTIs according to subjective or indirect measures, such as self-reported symptoms, visits to general practitioners, antibiotics use, or even school loss, through which viral and non-viral infections were unable to be differentiated [12–16]. The effects of gut microbiota modulation on tackling viral RTIs remain unclear. Therefore, we performed a systematic review focusing on viral RTIs whereby etiologies were confirmed by virology tests.

The aims of this systematic review were to (i) determine the efficacy of gut microbiota modulation using probiotics on outcomes of viral RTIs in clinical studies; and (ii) delineate the role of probiotics and the importance of commensal gut microbiota in protecting the host against viral RTIs in animal models.

Methods

Search strategy

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [17]. An electronic literature search was performed on Embase and Ovid MEDLINE using the following keyword combinations: ('virus' or 'Coronavirus') and 'infection' and ('microbiota' or 'microbiome' or 'probiotic' or 'prebiotic' or 'synbiotic'). The search was implemented without a starting date being applied but until 20 April 2020. The detailed searching strategy is provided in Additional file 1. Reference lists of original articles and relevant reviews were manually searched to identify additional studies for inclusion. After removal of duplicated references, initial screening of article titles and abstracts was undertaken by two independent investigators (HYS and XZ). Potential relevant articles were obtained in full text and reviewed independently. Disagreements were resolved through consensus and discussion with a third investigator (JWYM). Predefined criteria were used to determine eligibility for inclusion.

Selection criteria

We included interventional studies reporting the role of gut microbiota modulation on viral RTIs: (1) clinical studies on probiotics application in viral RTIs; (2) animal studies investigating the effects of gut microbiota manipulation on viral RTIs. Studies with clinical, virological, pathological or immunological outcomes were included. Studies were excluded if: (1) infecting organisms were not identified; (2) respiratory tract injury was caused by systemic viral infection (i.e. human immunodeficiency virus infection); (3) we were unable to separate viral RTIs from viral infections of other sites (i.e. gastrointestinal tract); (4) none of the outcomes (rate of RTIs, symptoms, viral load, respiratory pathology, virus-specific antibodies) were presented; (5) paper was published as conference abstract, review, letter, note, lecture, comment or editorial; (6) full texts were unavailable; (7) the paper was not in English language.

Data extraction

Two investigators (HYS and XZ) extracted data and entered it into a spreadsheet independently. A third investigator (WLL) evaluated the accuracy of this process. The collected data included the first author, country, study type, virus, subjects, sample size, age, sex, methodology of gut microbiota manipulation (details of strains used for intervention, administration form, administration dose, treatment duration, follow-up duration), outcomes and immune response.

Risk of bias assessment

All included clinical trials were independently assessed for bias using the Cochrane Handbook for Systematic Review of Interventions [18] by 2 investigators (HYS and XZ), with disagreements resolved by a third investigator (JWYM). Bias was assessed on selection (randomization, allocation concealment), performance (blinding of participants and personnel), detection (blinding of outcome assessment), attrition (incomplete outcome data), reporting (selective reporting), and other bias (e.g., funding).
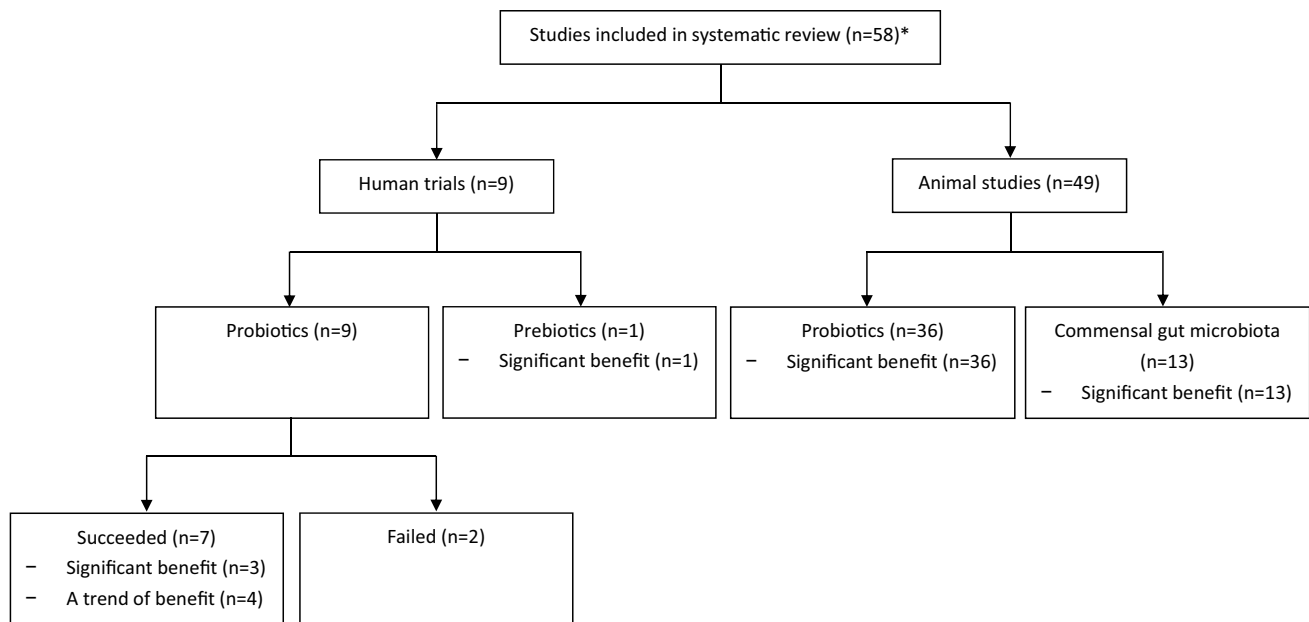
Results

Study selection

Overall, 1719 records were retrieved, and an additional six records were identified from reference lists of the related articles. After removal of 70 duplicates, 1655 records were screened according to the general criteria. Based on titles and abstracts, 1555 citations were rejected during the initial screen. Full texts of the remaining 100 articles were further reviewed for eligibility, and an additional 41 articles were excluded. Finally, our systematic review included 59 articles from 58 studies (Figs. 1 and 2).

Human trials

Nine randomized controlled trials (RCTs) [19–28] involving 1240 healthy individuals assessed the efficacy of probiotics or prebiotics in preventing viral RTIs (Table 1). The nine trials reported the results of probiotics in individuals of different age groups: five [19, 20, 22, 24, 26, 27] focused on adults
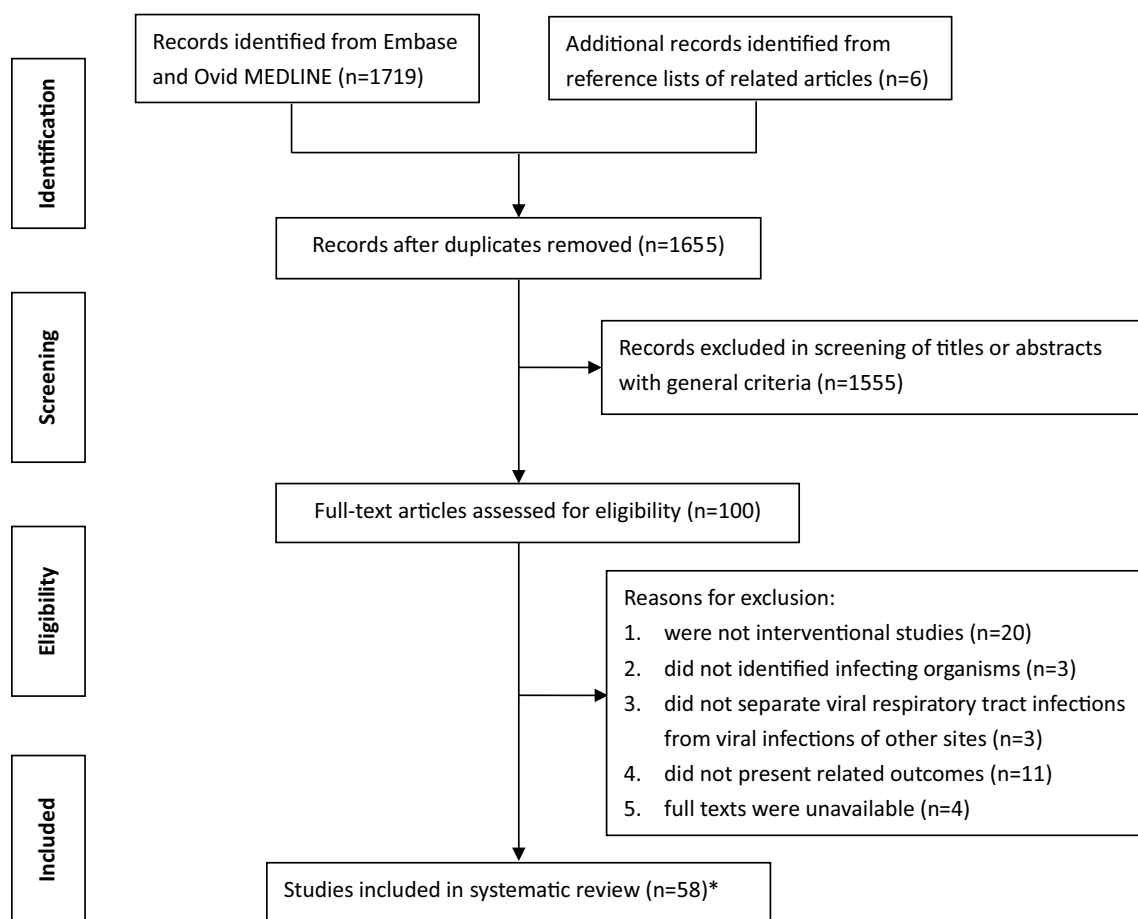
Fig. 1 Literature search and selection of included studies in this systematic review. * Fifty-nine articles from 58 studies

Studies included in systematic review (n=58)*

Human trials (n=9)

Animal studies (n=49)

Probiotics (n=9)

Prebiotics (n=1)
- Significant benefit (n=1)

Probiotics (n=36)
- Significant benefit (n=36)

Commensal gut microbiota (n=13)
- Significant benefit (n=13)

Succeeded (n=7)
- Significant benefit (n=3)
- A trend of benefit (n=4)

Failed (n=2)

Fig. 2 Categorization of included studies. * Fifty-nine articles from 58 studies

($n = 675$), two [21, 28] on elderly subjects ($n = 303$), one on children [23] ($n = 194$) and one on preterm infants (n = 68) [25]. *Lactobacillus* was the most commonly used probiotics [19, 21–25, 27, 28], followed by *Bifidobacterium* [20, 24] and *Lactococcus* [26]. One trial also evaluated the efficacy of prebiotics (galacto-oligosaccharide and polydextrose) in preventing viral RTIs [25].

Effect of probiotics in reducing risk of infection

Of the eight studies with viral infection rate reported, the protective effects of probiotics in reducing the infection risk were noted in six studies [20–22, 24, 25, 27], although statistical significance was not observed in four of them [20–22, 27]. In a small study, there was less frequent occurrence of picornaviruses (mainly rhinovirus) infection after three months of probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium animalis ssp. lactis* BB-12) intake (5/13 vs. 15/17 of control group, $p = 0.0069$) among military recruits [24]. Another study found that among preterm infants, the rate of viral RTIs was reduced in infants with administration of probiotic (*Lactobacillus rhamonosus* GG) (52.4%) than those on placebo (83.3%) during a 12-month follow-up [25]. Frequent (> 3 episodes) viral RTIs were less commonly found in probiotic group (9.5%) than placebo group (33.3%). The risk of rhinovirus infection was decreased in probiotic group (rate ratio [RR] 0.49; $p = 0.051$), compared with placebo group. In sensitivity analysis assuming that missing subjects remained healthy throughout the study period, the incidence of rhinovirus-induced episodes was significantly

lower in probiotic group, compared with that in placebo group (RR 0.43; $p = 0.041$).

Effect of probiotics on respiratory viral load

Three studies evaluated the impact of probiotics on viral load [19, 20, 25]. Two of these studies performed intranasal rhinovirus inoculation on healthy adults after 3 to 4 weeks of probiotics administration [19, 20]. The larger study with 115 subjects showed that pretreatment with 4 weeks of *Bifidobacterium* was associated with a significant reduction in nasal lavage virus titers ($p = 0.03$) and the proportion of subjects with virus shedding in nasal secretion was lower in the probiotic than placebo group (76% vs. 91%; $p = 0.04$) [20]. A separate small study showed a tendency towards a lower viral load in subjects pretreated with 3 weeks of *Lactobacillus* [19]. Among preterm infants, there were no significant differences on rhinovirus load between the *Lactobacillus rhamonosus* GG and placebo groups [25]. During symptomatic rhinovirus episodes, the median time for virus eradication was 10–15 days in the probiotic group, whereas it was more than 15 days in the placebo group, although the difference did not reach a statistical significance [25].

Effect of probiotics on clinical symptoms of viral infections

Four studies investigated the effect of probiotics on clinical symptoms caused by viral RTIs in a total of 441 subjects [20, 22, 23, 25]. One study showed a trend towards improved symptoms during the five days following rhinovirus

Table 1 Clinical studies of probiotics and risk of viral RTIs

First author, year of publication	Country	Participants	N/ male:female	Infected virus	Virus inoculation	Probiotic(s); rebiotic(s)	Probiotic dose; prebiotic dose	Administration form	Treatment duration	Follow-up duration	Viral infection rate	Viral load	Symptom score
Yamamoto, 2019 [28]	Japan	Elderly	107/33:74	IFV	No	*Lactobacillus delbrueckii bulgaricus*	$1.8–3.5\times10^{10}$ CFU/d	Yogurt beverage	12wk	12wk	–	–	–
Wang, 2018 [21]	Canada	Elderly	196/64:132	IFV, HRV, RSV, metapneumovirus, parainfluenza	No	*Lactobacillus rhamnosus GG*	2×10^{10} CFU/d	Capsule	6mo	6mo	→	–	–
Shida, 2017 [27]	Japan	Healthy adults	96/96:0	IFV	No	*Lactobacillus casei Shirota*	1×10^{11} CFU/d	Milk formula	12wk	12wk	→	–	–
Turner, 2017 [20]	United States	Healthy adults	115/43:72	HRV (28d after probiotic/placebo intervention)	Yes	*Bifidobacterium animalis lactis*	2×10^{9} CFU/d	Dissolved powder	33d	28d	→	↓*	NS
Tapiovaara, 2016 [19]	United States	Healthy adults	59/21:38	HRV (3w after probiotic/placebo intervention)	Yes	*Lactobacillus rhamnosus GG*	1×10^{9} CFU/d	Dissolved powder	6wk	3wk	-	→	–
Kumpu, 2015 [22]	United States	Healthy adults	59/21:38	HRV (3w after probiotic/placebo intervention)	Yes	*Lactobacillus rhamnosus GG*	1×10^{9} CFU/d	Dissolved powder	6wk	3wk	→	–	→
Sugimura, 2015 [26]	Japan	Healthy adults	213/92:121	IFV	No	*Lactococcus lactis*	1×10^{11} CFU/d	Yogurt beverage	10wk	10wk	NS	–	–

Table 1 (continued)

First author, year of publication	Country	Participants	N/ male:female	Infected virus	Virus inoculation	Probiotic(s); rebiotic(s)	Probiotic dose; prebiotic dose	Administration form	Treatment duration	Follow-up duration	Viral infection rate	Viral load	Symptom score
Lehtoranta, 2014 [24]	Finland	Healthy adults	192/-	IFV, RSV, parainfluenza viruses, adenovirus, human metapneumovirus, coronaviruses, picornaviruses, human bocavirus	No	*Lactobacillus rhamnosus GG; Bifidobacterium animalis lactis*	1×10^{10} CFU/d(*L. rhamnosus*); 4×10^9 CFU/d(*B. lactis*)	Chewing tablet	90d/150d	90d/150d	↓*	–	–
Luoto, 2014 [25]	Finland	preterm infants (1–3d)	68/44:24	IFV, HRV, RSV, enteroviruses, adenovirus, coronaviruses, human metapneumovirus, parainfluenza virus, human enterovirus, human bocavirus	No	*Lactobacillus rhamnosus GG; polydextrose and galactooligosaccharides*	1×10^9 CFU/d(d1–d30), 2×10^9 CFU/ d(d31–d60); 1×600 mg/d(d1–d30), 2×600 mg/d(d31–d60)	Milk formula	57d	12mo	↓*	↓(viral eradication time)	NS

Table 1 (continued)

First author, year of publication	Country	Participants	N/ male:female	Infected virus	Virus inoculation	Probiotic(s); prebiotic(s)	Probiotic dose; prebiotic dose	Administration form	Treatment duration	Follow-up duration	Viral infection rate	Viral load	Symptom score
Kumpu, 2013 [23]	Finland	children (2–6 Y/O)	194/103:91	IFV, HRV, RSV, parainfluenza virus, enterovirus, human bocavirus, adenovirus	No	*Lactobacillus rhamnosus GG*	1×10^8 CFU/d	Milk formula	28wk	28wk	NS	–	NS

IFV, influenza virus; HRV, human rhinovirus; RSV, respiratory syncytial virus; NS, no significant difference

*Significant difference

inoculation in probiotic group [22], the overall results of the four studies failed to identify a significant protective effect of probiotics on alleviating the severity of viral infection-induced RTI symptoms.

Effect of prebiotics in the prevention of viral infections

Only one study investigated the effects of prebiotics (galacto-oligosaccharide and polydextrose) in the prevention of viral RTIs [25]. Among preterm infants, prebiotics significantly reduced the risk of viral RTIs (39.1% vs. 83.3%, $p = 0.005$) and frequent viral RTIs (0% vs. 33.3%, $p = 0.005$), compared with placebo during a 12-month follow-up. The incidence of rhinovirus infection was significantly lower in prebiotic group than that in placebo group (RR 0.31, $p = 0.003$). In sensitivity analysis, assuming that missing subjects remained healthy throughout the study period, the incidence of rhinovirus-induced episodes was significantly lower in prebiotic group, compared with placebo group (RR 0.29, $p = 0.010$). There was no significant difference on rhinovirus load between the prebiotic and placebo groups. Among symptomatic patients, the median time for virus eradication was shorter in the prebiotic group (10–15 days) than that (15 days) in the placebo group, although the difference did not reach a statistical significance. The severity of clinical symptoms was not significantly different between the prebiotic and placebo groups.

Risk of bias assessment

Summary of the risk of bias for the clinical studies was demonstrated in Table 2. There were 4 studies (5 articles) [19, 22, 23, 26, 27] at unclear risk for selection bias (did not describe the methods used to generate random sequence or conceal the allocation sequence in sufficient details). The risk of performance bias was high in one study [27] (lack of identical placebo). For 7 studies (8 articles) [19–25, 28], there was unclear risk of attribution bias (missing data on the main outcome, no formal sample size calculation or unmet predefined sample size). There were 7 studies (8 articles) [19, 21–23, 25–28] at unclear risk of other bias because the studies were funded by of the probiotic manufacturer or distributor, or the authors were employed by the provider of the study product.

Animal studies

Probiotics and outcomes of viral RTIs in animal models

A total of 36 studies investigated the effects of oral administration of probiotics on outcomes of viral RTIs in animal models (Table 3). Probiotics were administered prior to viral

Table 2 Risk of bias summary for the randomized controlled trials in humans

First author, year of publication	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Yamamoto, 2019 [28]	L	L	L	L	U	L	U
Wang, 2018 [21]	L	L	L	L	U	L	U
Shida, 2017 [27]	U	U	H	L	L	L	U
Turner, 2017 [20]	L	L	L	L	U	L	L
Tapiovaara, 2016 [19]	L	U	L	L	U	L	U
Kumpu, 2015 [22]	L	U	L	L	U	L	U
Sugimura, 2015 [26]	L	U	L	L	L	L	U
Lehtoranta, 2014 [24]	L	L	L	L	U	L	L
Luoto, 2014 [25]	L	L	L	L	U	L	U
Kumpu, 2013 [23]	L	U	L	L	U	L	U

L, low risk; H, high risk; U, unclear risk

infection in all of the studies. The majority of the studies (n = 28) used *Lactobacillus* [9, 29–55], followed by *Bifidobacterium* [56–58], *Enterococcus* [59–61] or *Lactococcus* [62, 63]. Thirty-one studies investigated the outcome of probiotics in influenza virus [9, 29, 32–49, 51–53, 55–61, 63], four studies targeted respiratory syncytial virus (RSV) [30, 31, 36, 54], whilst the remaining studies focused on parainfluenza virus [62] and avian influenza virus [50].

Probiotics improved outcomes of mice infected with influenza virus

Twenty-two studies reported the effect of probiotics on survival after virus challenge [9, 32, 33, 35, 37, 42–49, 51, 53, 56–61, 63]. Amongst the 22 studies, 20 showed decreased mortality rates [35, 37, 42, 46–49, 51, 53, 56–61, 63] or prolonged survival time [33, 43–45] in mice, which were given probiotics. Five studies revealed a dose-dependent manner of *Lactobacillus* in improving survival [35, 44, 45, 47, 49]. One study found that live *Lactobacillus spp.* were superior to the inactivated ones in increasing survival rate [35].

Twenty-one studies evaluated the impact of probiotics on infection-induced symptoms [9, 29, 32–34, 37–42, 47–49, 53, 55–57, 59, 60, 63]. Being the most commonly reported symptom, weight loss was significantly mitigated in mice treated with probiotics in 18 studies [29, 32–34, 37, 39–42, 47–49, 55–57, 59, 60, 63]. Eleven studies scaled the symptoms using clinical scores based on fur appearance, eyelid, behavior or other indices, such as breath and body temperature, and all of these studies demonstrated significant

improvement in mice given probiotics [9, 29, 34, 37–39, 41, 53, 55–57].

Twenty-five studies investigated the effect of probiotics on influenza viral load. Virus titers were lower in 23 studies tested in lungs [9, 33, 35–42, 44, 45, 47–49, 55, 56, 59, 63], bronchoalveolar lavage fluid (BALF) [40, 46, 47, 57], and nasal washings [52, 53] of mice pretreated with *Lactobacillus* [9, 32, 33, 35–49, 52, 53, 55], *Bifidobacterium* [56, 57], *Enterococcus* [59] or *Lactococcus* [63]. A dose-dependent manner of *Lactobacillus* was also observed in suppressing viral replication in lungs [40, 44, 49].

Immune responses underlying the effects of probiotics on mice infected with influenza virus

Seventeen studies discussed the intimate involvement of immune cells in probiotic-mediated protection against influenza virus infection [27, 29, 31, 33, 39, 46, 48, 52, 53, 55, 57, 58, 62, 64–67]. In particular, studies reported increased natural killer (NK) cell activities [27, 39, 46, 52, 53, 57, 64, 67], or decreased infiltrating macrophages and neutrophils in BALF [33] upon probiotic administration. Meanwhile, two studies with *Lactobacillus* or *Lactococcus* administration dramatically increased the recruitment of dendritic cells (DCs) [29, 62].

Twelve studies reported the contribution of interferon-α/β (IFN-α/β) to immune defenses against viral infections triggered by probiotic administration [9, 26, 30, 34, 45, 54, 57, 62, 68–71]. Of these, studies regard to *Lactobacillus* [9, 30, 34, 45, 54], *Lactococcus* [26, 62], *Bifidobacterium* [57],

Table 3 Animal studies of probiotics and outcomes of viral RTIs

First author, year of publication	Country	Infected virus	Animal	Probiotic(s)	Live/killed	Dose/concentration	Treatment time (before infection)	Intervention duration	FU duration	Mortality	Symptoms	Viral load	Severity of respiratory pathology	Viral-specific antibodies
Ermolenko, 2019 [60]	Russia	IFV	Mice	*Enterococcus faecium*	–	1×10^7 CFU/d	1d	11d	15d	↓*	Weight loss:↓*	-	–	–
Mahooti, 2019 [58]	Iran	IFV	Mice	*Bifidobacterium bifidum*	–	2×10^8 CFU	21d	21d (7 times)	14d	↓*	–	–	–	IgG↑*
Takahashi, 2019 [32]	Japan	IFV	Mice	*Lactobacillus delbrueckii bulgaricus*	Live	1.14×10^8 CFU/d	22d	35d	14d	NS	Weight loss:↓*	NS	–	IgA↑* IgG↑*
Belkacem, 2018 [29]	France	IFV	Mice	*Lactobacillus paracasei*	–	2×10^8 CFU/d	7d	17d	9d	–	Weight loss:↓*; symptom scores:↓*	–	–	–
Park, 2018 [49]	Korea	IFV	Mice	*Lactobacillus plantarum*	Heat–killed	1×10^8 to 5×10^{12}/g, 0.05 mg or 10 mg dose in 200μL/d	14d	28d	14d	↓*	Weight loss:↓*	↓*	–	–
Belkacem, 2017 [55]	France	IFV	Mice	*Lactobacillus paracasei*	–	2×10^8 CFU/d	7d	17d	10d	–	weight loss:↓*; clinical scores:↑*#	↓*	↓*	–
Chen, 2017 [59]	Taiwan	IFV	Mice	*Enterococcus faecalis*	Live, heat–killed	8.5×10^{10} CFU/kg/d	12d	12d	10d	↓*	Weight loss:↓*	↓*	→	–
Song, 2016 [51]	Korea	IFV	Mice	*Lactobacillus rhamnosus*	Live	1×10^9 CFU/mL, 0.3 mL	14d	34d	20d	↓*	–	–	↓*	–
Kawahara, 2015 [57]	Japan	IFV	Mice	*Bifidobacterium longum*	–	2.0×10^9 CFU in 200uL/d	14d	17d	12d	↓*	Weight loss:↓*; symptom scores:↓*	↓*	↓*	–
Kikuchi, 2014 [42]	Japan	IFV	Mice	*Lactobacillus plantarum*	Heat–killed	120 mg LAB/d	28d	4w	10d	↓*	Weight loss:↓*	↓*	–	IgA: NS
Nakayama, 2014 [47]	Japan	IFV	Mice	*Lactobacillus gasseri*	–	1.0×10^8, 1.0×10^9 or 1.6×10^9 CFU/d	21d	42d	21d	↓*	Weight loss:↓*	↓*	→	–

Table 3 (continued)

First author, year of publication	Country	Infected virus	Animal	Probiotic(s)	Live/killed	Dose/concentration	Treatment time (before infection)	Intervention duration	FU duration	Mortality	Symptoms	Viral load	Severity of respiratory pathology	Viral-specific antibodies
Waki, 2014 [34]	New Zealand	IFV	Mice	*Lactobacillus brevis*	Live, lyophilized	1×10^9 CFU/d	14d	14d	7d	–	Weight loss:↓*; symptom scores:↓*	–	–	IgA↑*
Zelaya, 2014 [36]	Japan	IFV, RSV	Mice	*Lactobacillus rhamnosus*	–	10^8 cells/d	5d	5d	5d	–	–	↓*	–	–
Goto, 2013 [9]	Japan	IFV	Mice	*Lactobacillus acidophilus*	Live, heat–killed	10 mg/d (live bacteria: 100 mg/mL, 4×10^{10} CFU/ml; heat–killed bacteria: 33 mg/mL)	15d	21d	6d	NS	Weight loss:NS; symptom scores:↓*	↓*	↓*	IgG↓* IgA: NS
Kechaou, 2013 [41]	France	IFV	Mice	*Lactobacillus plantarum*	Live	1.0×10^9 CFU/d	10d	24d	14d	–	Weight loss:↓*; symptom scores:↓*	↓*	–	–
Kiso, 2013 [43]	Japan	IFV	Mice	*Lactobacillus pentosus*	Heat–killed	10 mg/d (10^{10} cells in 200 mL buffer saline)	21d	5w	2w	↓*	–	NS	NS	–
Park, 2013 [48]	United States	IFV	Mice	*Lactobacillus plantarum*	Live	10^9 or 10^8 CFU in 200 uL/d	10d	24d	14d	↓*	Weight loss:↓*	↓*	–	–
Iwabuchi, 2012 [37]	Japan	IFV	Mice	*Lactobacillus paracasei*	Heat–killed	1 mg/0.2 mL/d (1 mg contained 1.9×10^6 microorganisms)	14d	19d	6d	→	Weight loss:↓*; symptom scores:↓*	↓*	↓*	–
Kawase, 2012 [39]	Japan	IFV	Mice	*Lactobacillus gasseri*	Heat-killed	10 mg/d	14d	19d	20d	–	Weight loss:↓*; symptom scores:↓*	↓*	↓*	–
Kondoh, 2012 [61]	Japan	IFV	Mice	*Enterococcus faecalis*	Heat-killed	15 mg/d	7d	27d	21d	↓*	–	–	↓*	–
Maruo, 2012 [63]	Japan	IFV	Mice	*Lactococcus lactis cremoris*	–	$1.5–3.8 \times 10^8$ CFU/ml, 100uL/d	8d	12d	14d	↓*	Weight loss:↓*	↓*	–	–

Table 3 (continued)

First author, year of publication	Country	Infected virus	Animal	Probiotic(s)	Live/killed	Dose/concentration	Treatment time (before infection)	Intervention duration	FU duration	Mortality	Symptoms	Viral load	Severity of respiratory pathology	Viral-specific antibodies
Youn, 2012 [35]	Korea	IFV	Mice	Lactobacillus spp. (L. rhamnosus, L. plantarum, L. fermentum, L. brevis)	Live, heat-killed	10^8, 10^7, 10^6 or 10^5 CFU/d	21d	21d	14d	↓*	–	↓*	–	–
Iwabuchi, 2011 [56]	Japan	IFV	Mice	Bifidobacterium longum	Live, lyophilized	2.0×10^9 CFU/0.2 mL/d	14d	19d	6d	→	Weight loss:↓*; symptom scores:↓*	↓*	→*	–
Kawashima, 2011 [40]	Japan	IFV	Mice	Lactobacillus plantarum	Heat-killed	0.011, 0.21 or 2.1 mg/d	7d	14d	14d	–	Weight loss:↓*	↓*	–	IgA↑*
Kobayashi, 2011 [44]	Japan	IFV	Mice	Lactobacillus pentosus	Heat-killed	0.2, 0.4, 2, or 10 mg/d	21d	3w	2w	↓*	–	↓*	–	IgA↑* IgG↑*
Nagai, 2011 [46]	Japan	IFV	Mice	Lactobacillus delbrueckii bulgaricus	–	Yogurt 0.4 mL/d (3.5×10^8 cfu/g of L. bulgaricus, 6.8×10^8 cfu/g of S. thermophilus and 20 ug of polysaccharides)	21d	27d	21d	↓*	–	↓*	–	IgA↑* IgG↑*
Takeda, 2011 [33]	Japan	IFV	Mice	Lactobacillus plantarum	Heat-killed	20 mg, twice daily	2d	9d	14d	↓*	Weight loss:↓*	↓*	→	–
Kawase, 2010 [38]	Japan	IFV	Mice	Lactobacillus rhamnosus GG and Lactobacillus gasseri	Lyophilized	10 mg, in 200 µL/d	14d	19d	20d	–	Weight loss:NS; symptom scores:↓*	↓*	↓*	–
Maeda, 2009 [45]	Japan	IFV	Mice	Lactobacillus plantarum	Heat-killed	3 mg/kg/d, 15 mg/kg/d, 75 mg/kg/d or 100 mg/kg/d	7d	14d	14d	↓*	–	↓*	–	–

Table 3 (continued)

First author, year of publication	Country	Infected virus	Animal	Probiotic(s)	Live/killed	Dose/concentration	Treatment time (before infection)	Intervention duration	FU duration	Mortality	Symptoms	Viral load	Severity of respiratory pathology	Viral-specific antibodies
Yasui, 2004 [53]	Japan	IFV	Mice	Lactobacillus casei Shirota	Live	10^8 CFU/d, 5 times/week	before virus infection	3w (17 times)	17d	↓*	Symptom scores:↓*	↓*	–	–
Hori, 2002 [52]	Japan	IFV	Mice	Lactobacillus casei Shirota	Heat-killed	–	before virus infection	4 m	3d	–	–	↓*	–	–
Eguchi, 2019 [30]	Japan	RSV	Mice	Lactobacillus gasseri	Live, heat-killed, lyophilized	2×10^9 CFU/d	21d	25d	4d	–	Weight loss:↓*	↓*	–	–
Fujimura, 2014 [31]	United States	RSV	Mice	Lactobacillus johnsonii	live, heat-killed	3.9×10^7 CFU/d	7d	–	8d	–	–	–	↓*	–
Chiba, 2013 [54]	Japan	RSV	Mice	Lactobacillus rhamnosus	–	10^8 cells/d	5d	5d	5d	–	weight loss:↓*	↓*	↓*	–
Jounai, 2015 [62]	Japan	Parainfluenza	Mice	Lactococcus lactis	heat-killed	1 mg/d	14d	29d	15d	↓*	Weight loss:↓*; symptom scores:↓*	–	↓*	–
Poorbaghi, 2014 [50]	Iran	Avian influenza virus	Chicks	Lactobacillus acidophilus	–	Probiotic: 10^9 CFU, prebiotic: 0.1%	before virus infection	Probiotic: D1 and D17; prebiotic: 34d	14d	–	–	↓*	–	–

IFV, influenza virus; RSV, respiratory syncytial virus; NS, no significant difference

* Significant difference

Clinical scores: high scores indicated improved symptoms (while lower scores indicated improved symptoms in other studies)

or *Enterococcus* [71] administration showed increased production of either IFN-α or IFN-β. Sixteen studies addressed the alteration of IFN-γ in respiratory virus infected mice administered with probiotics [26, 33, 39, 48, 51, 52, 54–58, 69, 71–74]. There are 12 studies declared the increase of IFN-γ after administration of probiotic *Lactobacillus* [30, 32, 33, 39, 48, 51, 52, 54, 55], *Bifidobacterium* [57, 58], or Bifico [73] (a probiotic mixture consisting *Bifidobacterium, Lactobacillus acidophilus*, and *Enterococcus*). Seven studies indicated the roles of TNF-α in assisting immune defense against influenza virus infection [33, 36, 45, 48, 51, 52, 57]. Only one study detected increased production of TNF-α by nasal lymphocytes with administration of *Lactobacillus* [52]. Conversely, 6 of the studies consistently reported decreased TNF-α level, with 4 of them were being administered with *Lactobacillus* [33, 48, 74], 2 of them with *Enterococcus* [61, 71], while one of them with *Bifidobacterium* [57].

Seventeen studies reported the roles of dominant interleukins (ILs) in protective immune responses [9, 29, 33, 36, 48, 51, 53–59, 61, 71, 73, 74]. Four studies demonstrated that administration of *Lactobacillus* led to immune regulatory responses by upregulating IL-10 [36, 54, 55, 74]. Eight of the studies reported decrease in IL-6 after administration of *Lactobacillus* [33, 48], *Bifidobacterium* [56–58], *Enterococcus* [59, 71], or Bifico [73]. More specifically, strain-specific effects were observed in animal studies conducted using *Lactobacillus rhamnosus* M21 and CRL1505, respectively [51, 54], as *Lactobacillus rhamnosus* M21 mainly induces IL-12 increase while *Lactobacillus rhamnosus* CRL1505 promotes secretion of anti-inflammatory cytokine IL-10.

Eight studies [9, 32, 34, 40, 42, 44, 46, 58] tested the levels of virus-specific antibodies. Five of them reported increased influenza virus-specific immunoglobulin A (IgA) levels in the BALF [34, 40, 44, 46], and immunoglobulin G (IgG) titers in BALF [40, 44, 46] and serum [40, 44, 58] were elevated in the probiotic (*Lactobacillus* or *Bifidobacterium*) group, compared with non-probiotic group. Probiotic feeding stimulated not only the production of influenza virus-specific IgA and IgG titers, but also accelerate their neutralization activities in serum and BALF in mice treated with oseltamivir [32]. One study revealed a dose-dependent manner of probiotics in stimulating influenza virus-specific IgA and IgG production [44].

Probiotics improved outcomes of mice infected with RSV

All of the four studies [30, 31, 36, 54] focused on RSV infection showed that *Lactobacillus* protected mice against RSV infection with alleviated body weight loss [30, 54], suppressed pulmonary RSV load [30, 36, 54], and milder pathological changes in lungs [31, 54]. Live probiotics were superior to inactivated probiotics in reducing pulmonary histopathological inflammation [31].

Commensal gut microbiota and the outcomes of viral RTIs in animal models

A total of thirteen studies investigated the influence of commensal gut microbiota on the outcomes of viral RTIs in animal models (Table 4). Of these studies, ten focused on influenza virus [8, 65, 66, 68, 73, 75–79], and the remaining studies were on RSV [80], parainfluenza virus [64] and avian influenza virus [81].

All of the ten studies focused on influenza virus demonstrated beneficial effects of commensal gut microbiota against viral infection [8, 65, 66, 68, 73, 75–79]. In one study, natural gut microbiota from wild mice protected the recipient laboratory mice against influenza virus infection, manifested as reductions in mortality, weight loss, lung viral titers and respiratory immune-mediated pathology [8]. Depletion of intestinal commensal bacteria resulted in increased mortality [65, 79], greater weight loss [65, 75, 79], higher virus load [65, 66, 68, 75, 76, 78, 79] and more severe inflammation in lungs [65, 73, 78] of animals encountering viral challenge. In particular, antibiotic treatment by either cocktail (ampicillin, gentamicin, metronidazole, neomycin, vancomycin) [65, 66] or single antibiotic neomycin [66] both reduced CD4$^+$ or CD8$^+$ T cells. On the contrary, restoration of gut microbiota by probiotics (Bifico) supplementation [73], fecal microbiota transplantation (FMT) [79] or even commensal fungi (*Candida albicans* or *Saccharomyces cerevisiae*) colonization [77] was able to alleviate the severity of viral infection deteriorated by gut dysbiosis.

Discussion

To the best of our knowledge, this work is the first systematic review to report the role of gut microbiota manipulation on the risk and outcomes of viral RTIs. We found that modulation of gut microbiota may prevent viral RTIs in humans. Animal studies showed that treatments with probiotics before viral challenge were effective in improving the outcomes of viral RTIs, in terms of reducing infection-induced mortality, mitigating symptoms, decreasing viral load and boosting host immunity against viral infection. Disturbance of gut microbiota deteriorated in viral RTIs, which could be reversed by microbiota restoration. Furthermore, we have provided a data-driven explanation on the mechanisms by which gut microbiota modulation could impact viral RTIs.

Probiotics have been widely used to normalize perturbed gut microbiota and confer health benefits on hosts [82]. To focus on viral infections, we only included studies with positive virological tests or specific respiratory virus inoculation. Among the 8 RCTs in humans reporting virus infection rate, 6 showed decreased rates of RTIs in the probiotic group, suggesting probiotics are potentially promising agents to

Table 4 Animal studies of commensal gut microbiota and the outcomes of viral RTIs

First author, year of publication	Country	Infected virus	Animal	Reason cause micriobiota change	Dose/concentration	Time of treatment initiation	Intervention duration	FU duration	Weight loss	Mortality	Viral load/virus shedding	Severity of respiratory pathology
Bradley, 2019 [79]	UK	IFV	Mice	Antibiotics and FMT	ABX: ampicillin(100 mg), vancomycin(100 mg), metronidazole(100 mg), gentamicin(100 mg) in 100 mL water; FMT: 200ul (D2-D5 post ABX treatment)	ABX: after virus infection; FMT: after ABX treatment	4wk (ABX)	–	ABX:↑*, FMT:↓*	ABX:↑*	ABX:↑*	
Fuglsang, 2018 [75]	Austrilia	IFV	Mice	Antibiotics	Ampicillin (0.5 mg/mL), gentamicin sulfate (0.5 mg/mL), metronidazole (0.5 mg/mL)	2wk before virus infection	2wk (ABX2), 3wk (ABX1)	28d	ABX:↑*	–	ABX:↑*	–
Pang, 2018 [78]	China	IFV	Mice	Antibiotics	0.30 g/kg/d	14d before virus infection	14d	5d	–	–	ABX:↑*	ABX:↑*
Yitbarek, 2018 [76]	Canada	IFV	Chickens	Antibiotics	Vancomycin(5 mg/mL), neomycin(10 mg/mL), metronidazole(10 mg/mL), amphotericin B(0.1 mg/mL), twice daily, and ampicillin (1 g/L, continuously provided)	d0–d15 before virus infection	15d	14d	–	–	ABX:↑*	–

Table 4 (continued)

Table 4 (continued)

First author, year of publication	Country	Infected virus	Animal	Reason cause micriobiota change	Dose/concentration	Time of treatment initiation	Intervention duration	FU duration	Weight loss	Mortality	Viral load/virus shedding	Severity of respiratory pathology
Yitbarek, 2018 [68]	Canada	IFV	Chickens	Antibiotics	ABX: Vancomycin(5 mg/mL), neomycin(10 mg/mL), metronidazole(10 mg/mL), amphotericin-B(0.1 mg/mL) 10 mL/kg, twice daily and ampicillin(1 g/L, continuously provided); probiotics: 10^9 CFU/d; FMT: 8 mg with 1×10^9 bacterial cells/d	ABX 12d and probiotics/FMT 4d before virus infection, ABX 16d before virus infection	20d (ABX); 4d (probiotics or FMT)	5d	—	—	ABX:↑* Probiotics:↓* FMT:↓*	—
Jiang, 2017 [77]	United States	IFV	Mice	Antibiotics and fungi	Fungi: 10^6 CFU/d; ABX: ampicillin(0.5 mg/mL), gentamicin(0.5 mg/mL), metronidazole(0.5 mg/mL), neomycin(0.5 mg/mL), vancomycin(0.25 mg/mL), fluconazole (0.5 mg/mL), mannan 10 mg every other day	Fungi: 17d before virus infection, mannan: every other day starting 1 day prior to virus infection	14d (fungi)	20d	—	ABX:↑* Fungi:↓*	—	—
Rosshart, 2017 [8]	United States	IFV	Mice	Ileocecal microbial communities	0.1 mL–0.15 mL	d14, d15, d16 of pregnancy	3d	18d	GM:↓*	GM:↓*	GM:↓*	GM:↓*
Wu, 2013 [73]	China	IFV	Mice	Antibiotics and probiotic	ABX: neomycin sulfate(6 mg/d); probiotic: Bifico(3.06 mg/d)	ABX: d1–d8 before virus infection; probiotic: d9–d12	12d	—	—	—	—	ABX:↑*, probiotic:↓*

Table 4 (continued)

First author, year of publication	Country	Infected virus	Animal	Reason cause micriobiota change	Dose/concentration	Time of treatment initiation	Intervention duration	FU duration	Weight loss	Mortality	Viral load/virus shedding	Severity of respiratory pathology
Abt, 2012 [65]	United States	IFV	Mice	Antibiotics	Ampicillin (0.5 mg/mL), gentamicin(0.5 mg/mL), metronidazole(0.5 mg/mL), neomycin(0.5 mg/mL), vancomycin(0.25 mg/mL)	2–4wk before viral infection	2–4wk + 12d	12d	ABX:↑*	ABX:↑*	ABX:↑*	ABX:↑*
Ichinohe, 2011 [66]	United States	IFV	Mice	Antibiotics	Ampicillin(1 g/L), vancomycin(500 mg/L), neomycin sulfate(1 g/L), and metronidazole(1 g/L)	4wk before virus infection	6wk	2wk	–	–	ABX:↑*	–
Lynch, 2018 [80]	Australia	RSV	Mice	Antibiotics	Vancomycin (0.25 g/L), neomycin (0.5 g/L), ampicillin (0.5 g/L), metronidazole (0.5 g/L)	d13 of embryonic	6wk	–	ABX:↑*	–	NS	ABX:↑* (pDC△ mice)
Grayson, 2018 [64]	United States	Paramyxoviral virus	Mice	Antibiotics	Streptomycin sulfate (0.5 g/250 mL)	14d before virus infection and during virus infection	14d	15d	–	ABX:↑*	ABX:↓(viral clearence)*	–
Figueroa, 2020 [81]	France	Avian influenza virus	Mice	Antibiotics	Vancomycin(80 mg/kg), neomycin(300 mg/kg), metronidazole(200 mg/kg), ampicillin(200 mg/kg), colistin(24 mg/kg), amphotericin B(2 mg/kg) daily	2wk before virus infection	21d	7d	–	–	ABX:↑*	NS

IFV, influenza virus; RSV, respiratory syncytial virus; ABX, antibiotics; FMT, fecal microbiota transplantation; GM, gut microbiota; NS, no significant difference

*significant difference

prevent viral RTIs. However, heterogeneity in studies had hindered direct comparison of individual study. Although current clinical studies did not show a significantly milder viral-induced symptoms in subjects on probiotics, animal studies illustrated a protective effect of *Lactobacillus* [9, 29–49, 51–54], *Bifidobacterium* [56–58], *Enterococcus* [59–61] and *Lactococcus* [62, 63] in alleviating the severity of viral infections. Mechanistically, probiotics could elicit protective responses against viral RTIs by inducing cytokine/chemokine production which further engage immune cells to modulate antiviral immunity, strengthening mucosal barrier through increasing mucin and tight junction molecules, etc. [83]. Probiotic-induced IFN-γ have functions in regulating both innate and adaptive immunity, making it powerful in macrophages activation and wide-spectrum antiviral defenses [84]. Also, majority of probiotics strengthen host immunity by utilizing IL-4, IL-10, IL-12, IL-18 as immune-response mediators while producing IL-1α/β, IL-6, and TNFα as pro-inflammatory factors, which in parallel depicted a preventative cytokine signature for evaluating probiotics [33, 48, 54, 57, 61, 71]. For example, oral administration of *Bifidobacterium longum* MM-2 elicit NK cell activation through upregulating pulmonary expressions of IFN-γ, IL-2, IL-12 and IL-18, which further result with anti-influenza virus responses [57], while *Enterococcus faecalis* FK-23 oral administration, which exhibited improved survival rate in mouse model challenged by influenza A virus, upregulated anti-inflammatory IL-10 in lung tissues [61]. Probiotic boosts adaptive immune response with increased production of viral-specific IgA and IgG which is beneficial in defending viral RTIs. Besides innate and adaptive immunity, gut microbiota metabolism regulates inflammation through specific commensal bacteria that consume non-digestible dietary fibers and generate metabolites short-chain fatty acids (SCFAs, notably acetate, propionate, as well as butyrate) engaging in favoring mucosal barrier functionalities [80, 85–87]. However, these studies are all based on animal models. The mechanisms of probiotics in human viral RTIs need further investigations.

Data from animal studies have been predictive for the outcomes in subsequent human studies using the same strains. Oral administration of *Lactobacillus delbrueckii bulgaricus* OLL1073R-1 significantly prolonged the survival, reduced weight loss, decreased viral load and increased the anti-influenza virus antibodies in mice [32, 46]. Evidence has shown that consuming yogurt fermented with *Lactobacillus delbrueckii bulgaricus* OLL1073R-1 may help prevent influenza A virus subtype H3N2 infection by increasing the production of H3N2-bound salivary IgA in the elderly [28]. *Lactococcus lactis* subsp. *lactis* JCM5805-fed mice showed a drastic improvement in survival rate, alleviation of infection related symptoms, and reduction in lung histopathology scores in parainfluenza virus infection [62]. A RCT on

healthy adults had shown that *Lactococcus lactis* subsp. *lactis* JCM5805 intake significantly reduced the cumulative incidence days of major symptoms of an influenza-like illness [26]. Mice studies evidenced the effects of different *Lactobacillus plantarum* strains in improving the outcomes of influenza virus infection [33, 35, 40–42, 45, 48, 49]. Oral administration of heat-killed *Lactobacillus plantarum* L-137 enhanced protection against influenza virus infection by stimulating type I interferon production in mice [45]. A RCT conducted in subjects with high psychological stress levels showed that the incidence of upper RTI symptoms was significantly lower in those treated with heat-killed *Lactobacillus plantarum* L-137 [88]. The mechanistic read-outs observed in mice could also be reproduced in humans. However, the overall effects of probiotics were much clearer in the animal studies than in humans, which may be explained by the more heterogenous setting including differences in environmental and host factors that are known to influence the gut microbiome (e.g. diet, drugs, co-morbidities, follow-up duration, immune status, etc.) in human studies.

The effects of probiotics are influenced by multiplex factors. Probiotics confer a health benefit when administered in adequate amounts [82]. *Lactobacillus rhamonosus* GG was the first patented strain belonging to the genus *Lactobacillus* with a large number of research data as the basis for its use in combating against RTIs in humans [89]. For preventing viral RTIs, *Lactobacillus rhamonosus* GG at a daily dose of 10^9 CFU or above appeared to be effective in human trials [21, 22, 24, 25], whereas a dose of 10^8 CFU daily did not show a significantly positive role [23]. The administration of *Lactobacillus* spp. was shown to protect against influenza virus infection in a dose-dependent manner in mice [35, 40, 44, 45, 47, 49]. It had been reported that the incidence and severity of upper RTIs negatively correlated with the duration of heat-killed *Lactobacillus plantarum* L-137 intake among healthy people [88]. Duration of probiotic treatment in the prevention of viral RTIs ranged from 33 days [20] to 28 weeks [23] in humans. However, due to scarcity of data and different types of formula used, we are unable to propose a minimal treatment dose and duration to ensure optimal outcome in fighting against viral RTIs at this stage. Although the overall safety profile of probiotics is satisfactory, it should be noted that probiotic use could be associated with a higher risk of infection and/or morbidity in vulnerable people [90]. There is an increasing interest in non-viable microorganism-inducible health benefits [91]. Animal studies demonstrated the beneficial effects of non-viable (mainly heat-inactivated) probiotics in viral RTIs [9, 33, 37, 39, 40, 42–45, 49, 52, 59, 61, 62], although their effects appeared to be inferior to the live ones [31, 35]. The effects of probiotics in eliciting cytokine profiles in human cells and stimulating host immune system against viral RTIs in mice are highly strain-specific [92]. Since the data of strains other

than *Lactobacillus rhamonosus* GG were fairly limited in human studies, comparison of the strain-specific effects against viral RTIs in humans remains unavailable. Probiotic mixtures have been proved to be more effective than single-strain probiotics in inhibiting pathogen growth, due to an additive and more synergetic multispecies probiotic consortium [93]. Taking advantage of this finding, FMT, which transplants the great mixture of healthy gut ecosystem to recipients, represents the most powerful strategy of restoring a balanced gut microbiota [94]. However, potential risk of infections caused by undetected or unmonitored pathogens remains to be a major concern for its broad applications [93]. To overcome this problem, Petrof et al. developed a stool substitute consisting of 33 different purified intestinal bacteria isolated from a healthy donor, and successfully treated patients with antibiotic-resistant *Clostridium difficile* colitis [95]. Accelerated by massively parallel sequencing, our knowledge of the composition and function of the human gut microbiota has dramatically extended the range of organisms with potential health benefits. The next-generation probiotics, such as *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Bacteroides fragilis* and *Bacteroides uniformis*, have been identified to exert anti-inflammatory effects in animal models [93, 96].

Prebiotics are substrates selectively utilised by host micro-organisms conferring a health benefit [97]. RCTs had shown that the use of prebiotics in infants could reduce the risk of RTIs [25, 98–100]. Luoto et al. demonstrated that both probiotics (*Lactobacillus rhamonosus* GG) and prebiotics (galacto-oligosaccharides and polydextrose) resulted in fewer episodes of viral RTIs, compared with placebo [25]. It is interesting to note that prebiotics tended to perform better than the probiotic in this trial. One possible reason might be the pre-existence of bifidobacteria-dominated infant gut microbiota, which strengthens the effect of "bifidogenic" prebiotics [99, 101]. A study on mice model had shown that specific dietary prebiotic oligosaccaharides potentiated immune response against viral RTI102.

The gut-lung axis has been proposed in the pathogenesis of certain respiratory diseases [103]. Evidence has implied a gut-lung crosstalk in viral RTIs. Gut microbiota influences the susceptibility and severity of viral RTIs. Natural gut microbiota exhibiting more diverse microbiomes balanced systemic and local inflammatory responses upon lethal influenza virus challenge, resulting in higher survival rates and a milder disease course, compared with gut microbiota of laboratory mice from a restrictive environment [8]. Mice with depletion of commensal gut microbiota had significantly worse outcomes of viral RTIs [64, 65, 73, 75, 78, 79], which was reversed by restoration of gut microbiota with probiotics [73], FMT [79], and even commensal fungi colonization [77]. Clinical observational studies have also evidenced the importance of healthy gut microbiota in protecting against

viral RTIs. Higher level of butyrate-producing gut bacteria significantly associated with less development of viral RTIs among kidney transplant recipients [104], and reduced risk of viral lower RTIs in patients post-allogeneic hematopoietic stem cell transplantation (HSCT) [105]. In another study involving patients underwent allogeneic HSCT and had viral RTIs post transplantation, the number of antibiotic-days was associated with progression to lower respiratory tract disease [106]. On the other hand, viral RTIs lead to gut microbiota alteration. Both influenza virus and RSV infections result in significant changes of gut microbiota in mice [72, 107, 108]. Influenza virus infection also resulted in decreased levels of SCFAs (the metabolic output of the gut microbiota) in both of the gut and blood in mice [86]. Oral administration of acetate protected mice against RSV infection [87]. Supplementation of acetate reduced lung pathology and improved survival rates of mice with influenza virus and *Streptococcus pneumoniae* superinfection [86].

COVID-19, caused by SARS-CoV-2, is a major public health crisis threatening the human world today. This novel coronavirus, along with the SARS-CoV and the MERS-CoV, belongs to *Betacoronavirus* genus [109]. Although patients typically present with fever and respiratory symptoms, the viruses can also affect the digestive system [110, 111]. Studies have reported frequencies of diarrhea ranging from 2.0 to 35.6%, 13.8 to 73.3% and 11.5 to 32.0% among patients with COVID-19, SARS and MERS, respectively [110]. Gut microbial dysbiosis has been identified in COVID-19 patients, characterized by enrichment of opportunistic pathogens and depletion of beneficial commensals [112, 113]. Gut microbiota alterations associated with disease severity. The severity of COVID-19 was positively correlated with the baseline abundance of *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi*, and inversely correlated with that of *Faecalibacterium prausnitzii* (an anti-inflammatory bacterium) [112]. Subjects with gut dysbiosis, such as elderly, immune-compromised patients and patients with other co-morbidities, tend to have more severe disease and poorer outcomes of COVID-19 [114]. It implies that gut microbiota modulation could potentially reduce disease severity. Most of the studies on gut microbiota modulation against viral RTIs were carried out on influenza virus as a prophylactic strategy, and no major evidence was available on its protective effect towards COVID-19, the importance of probiotics supplementation in COVID-19 treatment has been emphasized in the guidance given from China's National Health Commission [113]. However, not all probiotics are equivalent for efficacy [115]. A novel and more targeted approach to modulate gut microbiota as one of the therapeutic strategies for COVID-19 and its complications is much needed. In a recent study reported by d'Ettorre et al., among COVID-19 patients, oral administration of a probiotic mixture significantly reduced the risk of developing

respiratory failure, and a trend towards reduced rates of mortality [116]. The Chinese University of Hong Kong team has developed an oral gut microbiota modulating formulation against COVID-19. In a pilot study, this formulation significantly improved clinical symptoms and reduced pro-inflammatory immune markers in COVID-19 patients [117]. Viral infections predispose patients to secondary bacterial infections, which often result in a more severe clinical course [118]. Empirical antibiotics are sometimes used for the management of viral infection when secondary bacterial infection is a concern. However, Zuo et al. revealed that antibiotics use led to further loss of salutary symbionts and exacerbation of gut dysbiosis in COVID-19 patients [112]. Animal studies have also proved the adverse influence of antibiotics in viral RTIs [64, 65, 79]. These results suggest clinicians to avoid unnecessary antibiotics use in the treatment of viral RTIs.

Conclusion

In conclusion, previous studies shed a light on the influence of gut microbiota on the occurrence and outcomes of viral RTIs. Gut microbiota modification presented a potential prophylactic and therapeutic avenue against viral RTIs through boosting immunity of hosts. However, research in this field is still in its infancy. High-quality clinical trials, translational studies and mechanism investigations are urgently needed. Unlike animal models, humans are highly heterogeneous in terms of diet, age, genetic background and gut microbiota configuration, and therefore may respond differently to the same intervention. With the development of new technologies, individualized gut microbiota modification will become available to address specific consumer needs and issues. Next-generation probiotics specific to viral strains and individualized conditions of the hosts may become a promising therapy in the prevention and treatment of viral RTIs in the near future.

Declarations

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