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ORIGINAL RESEARCH

The influence of prebiotic or probiotic supplementation on antibody titers after influenza vaccination: a systematic review and meta-analysis of randomized controlled trials

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Background: Influenza infection is a common disease with a huge disease burden. Influenza vaccination has been widely used, but concerns regarding vaccine efficacy exist, especially in the elderly. Probiotics are live microorganisms with immunomodulatory effects and may enhance the immune responses to influenza vaccination.

Methods: We conducted a systematic review and meta-analysis to determine the influence of prebiotics/probiotics/synbiotics supplementation on vaccine responses to influenza vaccination. Studies were systematically identified from electronic databases up to July 2017. Information regarding study population, influenza vaccination, components of supplements, and immune responses were extracted and analyzed. Twelve studies, investigating a total of 688 participants, were included in this review.

Results: Patients with prebiotics/probiotics supplements were found to have higher influenza hemagglutination inhibition antibody titers after vaccination (for A/H1N1, 42.89 vs 35.76, mean difference =7.14, 95% CI =2.73, 11.55, P=0.002; for A/H3N2, 105.4 vs 88.25, mean difference =17.19, 95% CI =3.39, 30.99, P=0.01; for B strain, 34.87 vs 30.73, mean difference =4.17, 95% CI =0.37, 7.96, P=0.03).

Conclusion: Supplementation with prebiotics or probiotics may enhance the influenza hemagglutination inhibition antibody titers in all A/H1N1, A/H3N2, and B strains (20%, 19.5%, and 13.6% increases, respectively). Concomitant prebiotics or probiotics supplementation with influenza vaccination may hold great promise for improving vaccine efficacy. However, high heterogeneity was observed and further studies are warranted.

Keywords: influenza, influenza vaccine, probiotics, prebiotics, synbiotics, antibody titer, immune response

Introduction

Influenza is a common infectious disease with a huge disease burden worldwide. It is estimated to be responsible for 250,000–500,000 deaths annually, especially among the elderly.[1] Influenza vaccination prevents influenza infection. Usually, the influenza vaccine is composed of split virions with 2 A strains (A/H1N1 and A/H3N2) and 1 B strain (Victoria or Yamagata lineages). Influenza vaccines are widely used, but concerns regarding vaccine efficacy exist, especially in the elderly. In a meta-analysis published in 2012, the pooled efficacy was 59% in adults aged 18–65 years, and evidence of protection in the elderly was lacking.[2] Low vaccine efficacy leads to inadequate protection, breakthrough infection, and influenza-related morbidity and mortality.

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Efforts have been made to improve the immune responses to influenza vaccines, such as adding adjuvant supplements, nutritional interventions, or increasing the vaccine dose.[3,4] In summary, the efficacy of the current influenza vaccine is not satisfactory.

The human intestine is host to a vast variety of microbes. Probiotics are microorganisms that have beneficial properties for the host and are known to alter the intestinal microflora.[5,6] Prebiotics are defined as dietary components that stimulate the growth and metabolic activity of probiotics. Synbiotics are the combination of prebiotics and probiotics. Application of prebiotics/probiotics/synbiotics suppresses the growth of pathogenic bacteria and improves the intestinal barrier function, and is widely used in patients with gastrointestinal infections and inflammation.[7,8] In addition to the beneficial effects on the intestinal tract, probiotics also have immunomodulatory effects by inducing production of protective cytokines and suppressing pro-inflammatory cytokines.[9–12] Extraintestinal benefits of probiotics include immune regulations in allergic diseases, cardiovascular diseases, and suppression of tumor growth.[13–15] Adjuvant probiotic use in these diseases is a potential target for future development.

The beneficial properties of immune modulation that follow probiotics consumption may enhance the immune responses to influenza vaccines.[16–20] Several randomized controlled trials (RCTs) have been conducted to investigate the influence of probiotics on influenza vaccines, but the results were inconsistent and inconclusive. Therefore, we conducted this systematic review and meta-analysis to evaluate the impacts of prebiotics/probiotics/synbiotics on immune responses after influenza vaccination.

Materials and methods

Study design and study selection

This study was approved by the Ethics Committees of MacKay Memorial Hospital, Taiwan (IRB No: 16MMHIS174e) and conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols guidelines.[21] We systematically searched for all relevant articles in the following online databases: Embase, PubMed, the Cochrane Library, the Cumulative Index to Nursing and Allied Health, the Airiti Library, and the PerioPath Index to the Taiwan Periodical Literature in Taiwan, from the earliest record to July 2017. The Cochrane Collaboration Central Register of Controlled Clinical Trials, Cochrane Systematic Reviews, and ClinicalTrials.gov were manually searched for additional references. The key terms used for the search were "influenza vaccine", "probiotics", "prebiotics", and "synbiotics". Keywords were combined using Boolean searches and the search was made using keywords, Boolean operators, and MeSH descriptor. The detailed search strategy is enclosed as Box S1. Two authors (P-CS and S-JL) conducted the search independently, and disagreements were resolved through discussion with the third author (W-TL).

After the initial search, 2 independent reviewers (P-CS and T-LY) assessed the eligibility of each publication. The inclusion criteria of selected RCTs were as follows: 1) studies in adults; 2) inclusion of a control group in the study design; 3) use of influenza vaccination and supplementation of probiotics, prebiotics, or synbiotics in the intervention group; 4) reporting of at least 1 immunological response to influenza vaccination. We excluded the following: 1) articles irrelevant to the topic; 2) duplicate publications; 3) trials of a cross-over study design; and 4) studies in which the control arm received an effective intervention rather than a placebo.

Data extraction and quality assessment

Two authors (W-TL and T-LY) independently evaluated the quality assessment of all eligible articles using the Cochrane Review risk of bias assessment tool. We assessed the adequacy of randomization, allocation concealment, blinding methods, implementation of the intent-to-treat analysis, dropout rate, complete outcome data, selective data reporting, and other biases of each enrolled publication.

The articles were scrutinized, and data regarding study population, influenza vaccine components, protocols of probiotics consumption, details of vaccine immune responses, and adverse effects from the selected studies were extracted. Discrepancies between the 2 independent evaluations for potential articles were resolved through discussion and consensus. The primary outcome was the immunogenicity of influenza vaccination, presented as hemagglutination inhibition (HI) antibody titers. The HI antibody titer equals the maximum dilution capable of inhibiting the agglutination of guinea pig red blood cells, with the influenza viruses under standardized conditions.[22] Other comparative variables included the components of the vaccine and probiotics, the protocols of probiotics consumption, and the serious adverse effects.

Data synthesis and analysis

Immunogenicity data from all the studies were extracted, analyzed, and compared to determine differences in the efficacy of influenza vaccination in the groups receiving prebiotics/probiotics/synbiotics supplementation and the placebo groups. Due to significant (and expected) heterogeneity among the studies, a random effects model was employed.[23] The results were represented by a point estimate with a 95% CI. The heterogeneity across studies was tested using I^2 and Cochran's Q tests. A P-value <0.10 for chi-square testing of the Q statistic or an $I^2>50\%$ was considered as statistically significant heterogeneity.[24] A sensitivity analysis was performed by removing some studies to observe whether the action caused serious changes in the overall results. The potential publication bias was assessed by observing the symmetry of funnel plots and using Egger's test.[25] Review Manager (version 5.3.5) was used for our analyses.

Results

Description of studies and quality assessment

Of the 22 non-duplicate citations identified from the literature, 2 studies were not RCTs and 20 were ultimately assessed for eligibility (Figure 1). Finally, 11 publications with 12 RCTs were included in our qualitative synthesis after critical review (Table 1).[26–36] Two trials (a pilot and a confirmatory study) with different patient numbers, treatment protocols, and years of study were published in the same article.[34] Seven studies investigated the effects of probiotics, and five studies investigated the effects of prebiotics. One study investigating synbiotics was excluded, after critical review, for using a different outcome parameter.[37] The included studies were conducted in the USA, France, Japan, and the UK. In total, 780 patients were enrolled in these studies with female predominance (M:F =1:2.1). Five different probiotics and 5 different prebiotics were used in the intervention arm. The trivalent inactivated influenza vaccines (TIV) were used in most studies (10/12). Most of the included studies had a low bias, as shown by our quality assessment using the Cochrane assessment tool. The detailed quality assessment of each included study is shown in Table S1.

Data synthesis and meta-analysis

Ultimately, 688 patients were enrolled in our meta-analysis. By comparing the HI titers of strain A/H1N1 after influenza vaccination, we found a significantly higher HI titers in the probiotics/prebiotics group (42.89 vs 35.76, mean difference =7.14, 95% CI =2.73, 11.55, $P<0.001$, $I^2=96\%$) (Figure 2). For strain A/H3N2, similar increase in HI titers was observed (105.4 vs 88.25, mean difference =17.19,

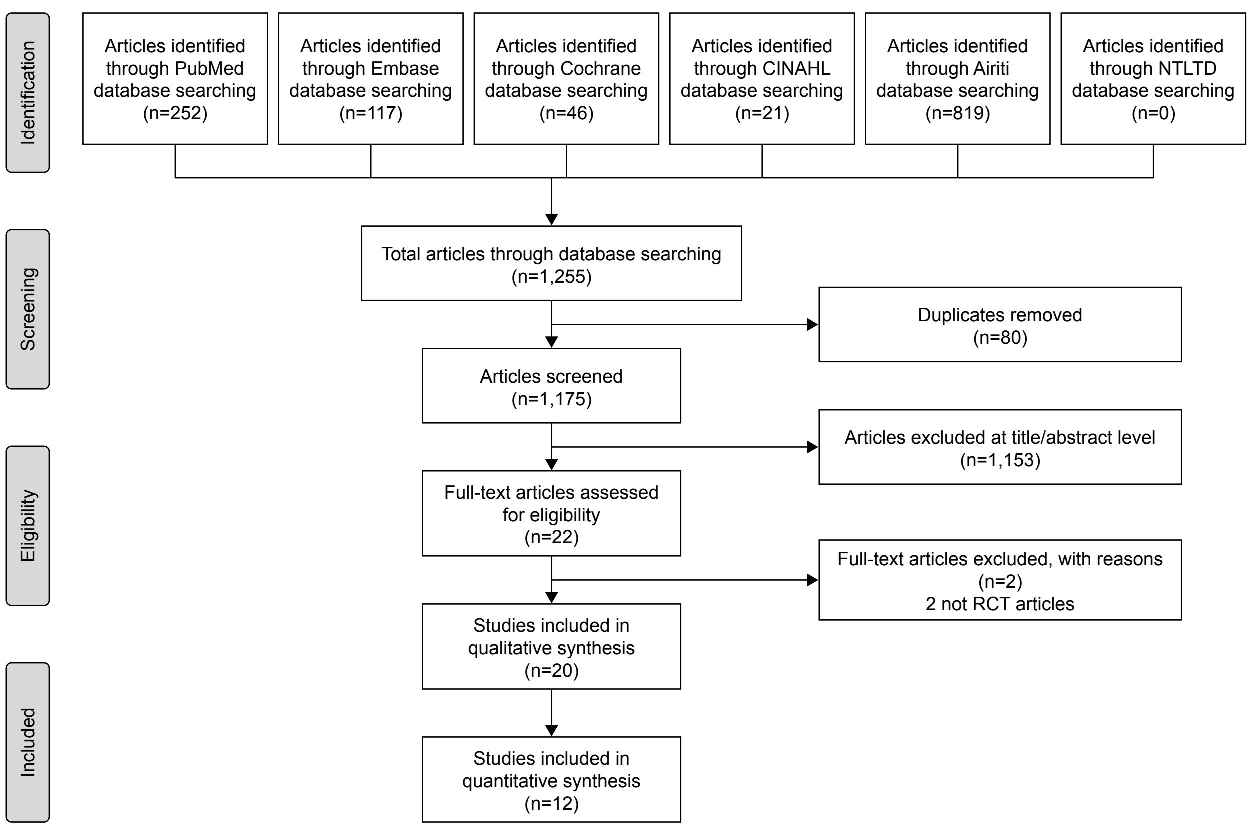

Figure 1 Flow diagram showing the selection of articles for review.
Abbreviations: CINAHL, Cumulative Index to Nursing and Allied Health; NTLTD, Networked Digital Library of Theses and Dissertations; RCT, randomized controlled trials.

Table 1 Characteristics of randomized controlled trials investigating the influence of prebiotics/probiotics on influenza vaccination

Reference, year	Country	Participants (M%:F%)	Age (years; mean [SD])	Supplement duration: total weeks (before/after vaccination)	Strains of supplements	Type of vaccine	Components of vaccine	Severe adverse events
Probiotics								
Olivares et al, 2007[67]	Spain	50 healthy adults (62%:38%)	33.00 (7.70)	4 (2/2)	*Lactobacillus fermentum* CECT5716 1×10^{10} CFU daily	TIV	H1N1: New Caledonia/20/99 H3N2: Fujian/411/2002 B: Shanghai/361/2002	Nil
French and Penny, 2009[68]	Australia	47 healthy adults (41%:59%)	31.55 (6.72)	6 (214)	*L. fermentum* VRI 003 1×10^{9} CFU	TIV	H1N1: New Caledonia/20/99 H3N2: Wisconsin/67/2005 B: Malaysia/2506/2004	30
Boge et al, 2009 (pilot)[34,a]	France	68 adults in nursing home (44%:56%)	83.64 (7.70)	7 (4/3)	*L. casei* DN-114 001 twice daily	TIV	H1N1: New Caledonia/20/99 H3N2: Wisconsin/67/2005 B: Malaysia/2506/2004	NR
Boge et al, 2009 (confirmed)[34,a]	France	222 elders in nursing home (33%:67%)	84.64 (6.72)	13 (4/9)	*L. casei* DN-114 001 twice daily	TIV	H1N1: New Caledonia/20/99 H3N2: California/7/2004 B: Shanghai/361/2002a B: Jiangsu/10/2003a	30
Namba et al, 2010[33,a]	Japan	27 elders in health care facility (11%:89%)	86.70 (6.60)	20 (3/17)	*Bifidobacterium longum* BB536 1×10^{11} CFU daily	TIV	H1N1: New Caledonia/20/99 H3N2: Wyoming/3/2003 B: Shanghai/361/2002	NR
Davidson et al, 2011[32,a]	USA	42 healthy adults (38%:62%)	33.30	4 (4/0)	*L rhamnosus GG* 1×10^{10} CFU twice daily	LAIV	H1N1: Solomon Islands/3/2006 H3N2: Wisconsin/67/2005 B: Malaysia/2506/2004	1
Van Puyenboreck et al, 2012[69]	Belgium	737 healthy adults in nursing home (25%:75%)	84.06	25 (3/22)	*L. casei Shirola* 6.5×10^{9} CFU twice daily	TIV	H1N1: Solomon Islands/3/2006 IVR-145 H3N2: Wisconsin/67/2005 Malaysia/2506/2004	NR
Rizzardini et al, 2012[70]	Italy	211 healthy adults (44%:56%)	33.2	6 (2/4)	*BB-12® (DSM15954)* 1×10^{9} CFU *L. casei* 431® (ATCC55544) daily	TIV	H1N1: Brisbane/59/2007 H3N2: Uruguay/716/2007 B: Florida/4/2006	Nil
Bosch et al, 2012[71]	Spain	60 adults in nursing home (NR)	65–85	12 (0/12)	*L. plantarum* CECT7315/7316 daily Group A: 5×10^{9} CFU Group B: 5×10^{8} CFU	TIV	H1N1: Solomon Islands/3/2006 IVR-145 H3N2: Wisconsin/67/2005 B: Malaysia/2506/2004	NR
Akatsu et al, 2013 (paper)[30,a]	Japan	45 enteral tube feeding adults (29%:71%)	81.70 (8.70)	12 (4/8)	*Bifidobacterium* strain, BB536 5×10^{10} CFU twice daily	TIV	H1N1: Brisbane/59/2007 H3N2: Uruguay/716/2007 B: Brisbane/60/2008	Nil

(*Continued*)

Table 1 (*Continued*)

Reference, year	Country	Participants (M%:F%)	Age (years; mean [SD])	Supplement duration: total weeks (before/ after vaccination)	Strains of supplements	Type of vaccine	Components of vaccine	Severe adverse events
Akatsu et al, 2013 (letter)[30,a]	Japan	15 adults in nursing home (47%:53%)	75.74 (7.22)	12 (3/9)	*L. paracasei* MoLac 1×10[11] CFU	TIV	A/H1N1: Brisbane/ 59/2007 A/H3N2: Uruguay/ 716/2007 B strain: Brisbane/60/2008	NR
Jespersen et al, 2015[72]	Germany, Denmark	1,104 healthy adults (41%:59%)	31.45	6 (3/3)	*L. casei* 43/(ATCC55544) l×10[9] CFU daily	TIV	A/H1N1: Califonia/ 7/2009 A/H3N2: Perth/16/2009 B strain: Brisbane/60/2008	5
Maruyama et al, 2016[26,a]	Japan	42 elders in nursing home (19%:81%)	87.15 (5.71)	6 (3/3)	*L. paracasei* MCC 1,849 1×10[11] CFU daily	TIV	A/H1N1: California/ 7/2009 pdm09 A/H3N2:Texas/50/2012 B strain: Massachusetts/2/2012 (Yamagata lineage)	Nil
Prebiotics								
Bunout et al, 2002[73]	Chile	66 healthy elders (similar%)	75.73	28 (1/27)	FOS (70% raftilose 30% raftiline) 2 sachets daily	TIV	A/H1N1: Caledonia A/H3N2: Moscow, Sydney B strain: Belgium (code 184–93)	3
Langkamp-Henken et al, 2004[36,a]	USA	66 healthy elders (47%:53%)	81.54 (1.35)	26 (2/24)	High oleic safflower oil, soybean oil, FOS, structured TG 8 oz daily	TIV	A/H1N1: Beijing/262/95 A/H3N2: Sydney/5/97 B strain: Yamanashi/166/98 (B/Beijing/184/93-like)	NR
Langkamp-Henken et al, 2006[35,a]	USA	157 frail elders in LTCI facilities (28%:72%)	83.36 (0.80)	10 (4/6)	Antioxidants, B vitamins, selenium, zinc, FOS, structured TG 240 mL daily	TIV	A/H1N1: Caledonia/ 20/99 A/H3N2: Panama/ 2007/99 B strain: Hong Kong/ 1434/2002	NR
Nagafuchi et al, 2015[28,a]	Japan	24 enteral tube feeding elders (46%:54%)	80.30 (9.80)	14 (4/10)	BGS (1.65 μg/ 100 kcal), DHNA, GOS (0.4 g/ 100 kcal), fermented milk products	TIV	A/H1NI: California/ 7/2009 A/H3N2: Victoria/ 210/2009 B strain: Brisbane/60/2008	Nil
Lomax et al, 2015[29,a]	UK	49 healthy adults (26%:74%)	54.98	8 (4/4)	50:50 mixture of long-chain inulin and oligofructose 8 g daily	TIV	A/H1N1: Brisbane/ 59/2007-like A/H3N2: Brisbane/ 10/2007-like B strain: Florida/4/2006-like	NR
Akatsu et al, 2016[27,a]	Japan	23 PEG-fed bedridden elders (13%:87%)	78.98 (9.09)	8 (4/4)	Heat-treated lactic acid bacteria fermented milk products, GOS 4 g/day, BGS 0.4 g/day	LAIV	A/H1N1: Solomon Islands/3/2006 A/H3N2: Hiroshima/ 52/2005 B strain: Malaysia/2506/2004	NR
Synbiotics								
Enani et al, 2017[37]	UK	112 healthy adults (NR)	18–35 60–85	8 (4/4)	*Bifidobacterium longum* l×10[9] CFU with GI-OS 8 g daily	TIV	A/H1N1: California/ 17/2009 pdm09 A/H3N2: Perth/16/2009 B strain: Brisbane/60/2008	NR

Note: [a]Included in meta-analysis.
Abbreviations: BGS, bifidogenic growth stimulator; CFU, colony-forming unit; DHNA, 1,4-dihydroxy-2-naphthoic acid; FOS, fructooligosaccharides; GOS, galacto-oligosaccharide; LAIV, live attenuated influenza vaccine; LTC, long term care facilities; Nil, no serious adverse events; NR, not reported; PEG, percutaneous endoscopic gastrostomy; TG, triglycerol; TIV, trivalent inactivated influenza vaccine.

Study or subgroup	Probiotics/prebiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI	Year
Probiotics									
Maruyama et al[26]	72.5	3.07	21	59.3	3.84	21	12.7	13.20 (11.10, 15.30)	
Akatsu et al (paper)[30]	15.85	1.17	23	14.45	1.23	22	13.0	1.40 (0.70, 2.10)	
Boge et al (pilot)[34]	44.72	11.38	44	38.21	12.2	42	11.2	6.51 (1.52, 11.50)	2009
Boge et al (confirmed)[34]	81.11	12.22	113	68.89	11.11	109	12.3	12.22 (9.15, 15.29)	2009
Namba et al[33]	15.85	3.98	13	19.95	2	14	12.6	–4.10 (–6.50, –1.70)	2010
Davidson et al[32]	26	8.8	19	25	8.1	20	11.0	1.00 (–4.32, 6.32)	2011
Akatsu et al (letter)[30]	5.01	1.7	8	2.29	1.47	7	12.8	2.72 (1.12, 4.32)	2013
Subtotal (95% CI)			**241**			**235**	**85.6**	**4.71 (0.53, 8.89)**	
Heterogeneity: τ^2=29.20; χ^2=179.13, *df*=6 (*P*<0.00001); I^2=97%									
Test for overall effect: *Z*=2.21 (*P*=0.03)									
Prebiotics									
Langkamp-Henken et al[36]	248.8	124.5	16	100	69.4	18	0.4	148.80 (79.88, 217.72)	
Langkamp-Henken et al[35]	79	11.5	52	60	9.4	40	11.6	19.00 (14.73, 23.27)	2006
Lomax et al[29]	518	1.103	22	1,103	3,398	21	0.0	–585.00 (–2,109.66, 939.66)	2015
Nagafuchi et al[28]	45.9	58.1	12	31.4	45.1	12	1.0	14.50 (–27.11, 56.11)	2015
Akatsu et al[27]	40	51.4	9	28	21	10	1.3	12.00 (–24.01, 48.01)	2016
Subtotal (95% CI)			**111**			**101**	**14.4**	**35.15 (0.31, 70.00)**	
Heterogeneity: τ^2=884.82; χ^2=14.40, *df*=4 (*P*=0.006); I^2=72%									
Test for overall effect: *Z*=1.98 (*P*=0.05)									
Total (95% CI)			**352**			**336**	**100**	**7.14 (2.73, 11.55)**	
Heterogeneity: τ^2=38.77; χ^2=253.11, *df*=11 (*P*<0.00001); I^2=96%									
Test for overall effect: *Z*=3.17 (*P*=0.002)									
Test for subgroup differences: χ^2=2.89, *df*=1 (*P*=0.09); I^2=65.4%									

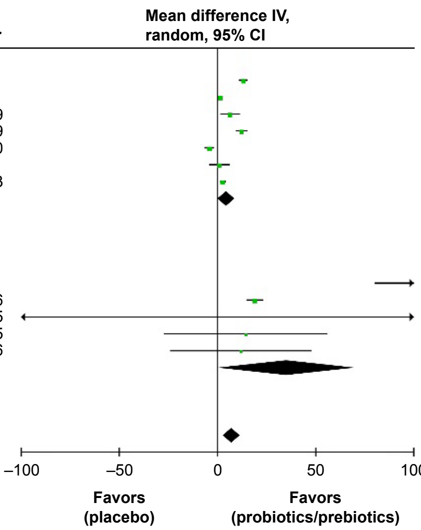

Figure 2 Forest plot of the HI titers of A/H1N1 strain after influenza vaccination between the prebiotic or probiotic group, and the placebo group.
Abbreviations: HI, hemagglutination inhibition; IV, inverse variance.

95% CI =3.39, 30.99, $P<0.001$, I^2=100%) (Figure 3). In patients with prebiotics/probiotics supplement, higher immune responses after influenza vaccination was noticed for strain B (34.87 vs 30.73, mean difference =4.17, 95% CI =0.37, 7.96, $P<0.001$, I^2=94%) (Figure 4). The percentages of increases were 20% (A/H1N1), 19.5% (A/H3N2), and 13.6% (B strain); the mean HI antibody titers are summarized in Table 2. Subgroup analysis of prebiotics and probiotics showed similar results. The heterogeneity was high in all analyses. We found no significant differences in serious adverse effects in either arm (Figure 5). The funnel plots were also assessed (Figures S1–S3).

Discussion

Our systematic review and meta-analysis support the beneficial effects of prebiotic/probiotic supplementation on humoral responses to influenza vaccination. We found that supplementation with pre- or probiotics enhanced the HI titers in all A/H1N1, A/H3N2, and B strains (20%, 19.5%, and 13.6% increases in HI antibody titers, respectively). Concomitant prebiotics/probiotics supplementation potentially improved the protection of influenza vaccination and decreased the subsequent risk of influenza-related morbidity and mortality. However, high heterogeneity was noted and further studies are warranted to consolidate this suggestion.

Study or subgroup	Probiotics/prebiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI	Year
Probiotics									
Maruyama et al[26]	145.01	3.34	21	171.25	3.92	21	11.0	–26.24 (–28.44, –24.04)	
Akatsu et al (paper)[30]	42.66	1.29	23	46.77	1.29	22	11.0	–4.11 (–4.86, –3.36)	
Boge et al (confirmed)[34]	114.12	24.71	113	89.41	23.53	109	10.7	24.71 (18.36, 31.06)	2009
Boge et al (pilot)[34]	104.07	12.2	44	63.41	12.2	42	10.8	40.66 (35.50, 45.82)	2009
Namba et al[33]	158.49	5.01	13	100	3.16	14	10.9	58.49 (55.30, 61.68)	2010
Davidson et al[32]	72	22	19	51	17.6	20	10.1	21.00 (8.46, 33.54)	2011
Akatsu et al (letter)[30]	7.41	1.38	8	2.95	1.51	7	11.0	4.46 (2.99, 5.93)	2013
Subtotal (95% CI)			**241**			**235**	**75.5**	**16.86 (0.87, 32.85)**	
Heterogeneity: τ^2=457.30; χ^2=2,286.59, *df*=6 (*P*<0.00001); I^2=100%									
Test for overall effect: *Z*=2.07 (*P*=0.04)									
Prebiotics									
Langkamp-Henken et al[36]	555	325	16	550	380	18	0.3	5.00 (–232.02, 242.02)	
Langkamp-Henken et al[35]	398	72	52	344	66.7	40	7.5	54.00 (25.54, 82.46)	2006
Nagafuchi et al[28]	14.5	10.8	12	13.6	10.7	12	10.5	0.90 (–7.70, 9.50)	2015
Lomax et al[29]	6,234	8,352	22	4,338	8,287	21	0.0	1,896.00 (–3,078.19, 6,870.19)	2015
Akatsu et al[27]	46.8	46	9	42	34.2	10	6.2	4.80 (–31.98, 41.58)	2016
Subtotal (95% CI)			**111**			**101**	**24.5**	**18.66 (–13.24, 50.56)**	
Heterogeneity: τ^2=637.86; χ^2=12.81, *df*=4 (*P*=0.01); I^2=69%									
Test for overall effect: *Z*=1.15 (*P*=0.25)									
Total (95% CI)			**352**			**336**	**100**	**17.19 (3.39, 30.99)**	
Heterogeneity: τ^2=451.29; χ^2=2,301.75, *df*=11 (*P*<0.00001); I^2=100%									
Test for overall effect: *Z*=2.44 (*P*=0.01)									
Test for subgroup differences: χ^2=0.01, *df*=1 (*P*=0.92); I^2=0%									

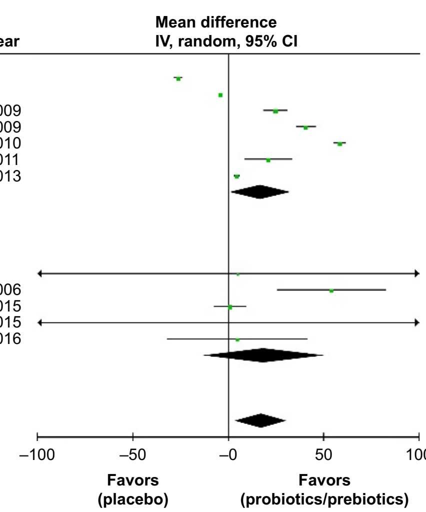

Figure 3 Forest plot of the HI titers of A/H3N2 strain after influenza vaccination between prebiotic or probiotic group, and placebo group.
Abbreviations: HI, hemagglutination inhibition; IV, inverse variance.

Study or subgroup	Probiotics/prebiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI	Year
Probiotics									
Akatsu et al (paper)[30]	11.75	1.17	23	14.45	1.23	22	14.1	–2.70 (–3.40, –2.00)	
Maruyama et al[26]	19.97	2.14	21	24.42	2.41	21	13.9	–4.45 (–5.83, –3.07)	
Boge et al (pilot)[34]	44.72	13.01	44	38.21	9.76	42	11.5	6.51 (1.66, 11.36)	2009
Boge et al (confirmatory)[34]	100	24.42	113	73.26	22.09	109	10.4	26.74 (20.62, 32.86)	2009
Namba et al[33]	10	5.01	13	15.85	3.98	14	12.7	–5.85 (–9.28, –2.42)	2010
Davidson et al[32]	31	4	19	25	4.5	20	13.2	6.00 (3.33, 8.67)	2011
Akatsu et al (letter)[30]	3.63	1.45	8	2	1.74	7	13.8	1.63 (–0.00, 3.26)	2013
Subtotal (95% CI)			**241**			**235**	**89.6**	**3.04 (–0.77, 6.85)**	
Heterogeneity: τ^2=23.66; χ^2=170.39, *df*=6 (*P*<0.00001); I^2=96%									
Test for overall effect: *Z*=1.56 (*P*=0.12)									
Prebiotics									
Langkamp-Henken et al[36]	460	220	16	450	183.3	18	0.1	10.00 (–127.08, 147.08)	
Langkamp-Henken et al[35]	336	61.3	52	279	56.4	40	2.1	57.00 (32.85, 81.15)	2006
Nagafuchi et al[28]	13.5	23.5	12	17.8	13	12	4.3	–4.30 (–19.49, 10.89)	2015
Lomax et al[29]	276	472	22	139	187	21	0.0	137.00 (–75.83, 349.83)	2015
Akatsu et al[27]	24.5	22	9	16.4	13.2	10	3.8	8.10 (–8.44, 24.64)	2016
Subtotal (95% CI)			**111**			**101**	**10.4**	**20.68 (–9.07, 50.42)**	
Heterogeneity: τ^2=642.99; χ^2=19.29, *df*=4 (*P*=0.0007); I^2=79%									
Test for overall effect: *Z*=1.36 (*P*=0.17)									
Total (95% CI)			**352**			**336**	**100**	**4.17 (0.37, 7.96)**	
Heterogeneity: τ^2=26.46; χ^2=196.31, *df*=11 (*P*<0.00001); I^2=94%									
Test for overall effect: *Z*=2.15 (*P*=0.03)									
Test for subgroup differences: χ^2=1.33, *df*=1 (*P*=0.25); I^2=24.7%									

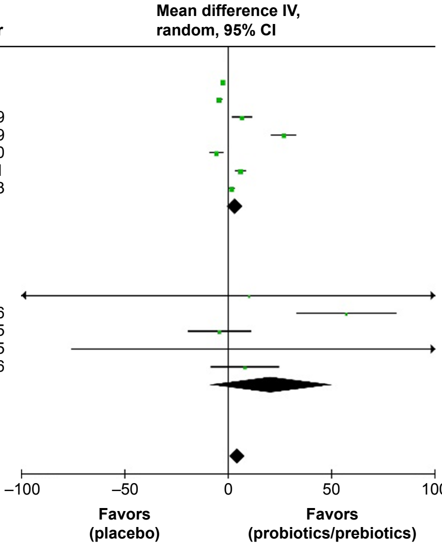

Figure 4 Forest plot of the HI titers of B strain between prebiotic or probiotic group, and placebo group.
Abbreviations: HI, hemagglutination inhibition; IV, inverse variance.

Influenza is highly contagious and virulent. Despite widespread use of influenza vaccination, it remains an important health threat. Currently, the effectiveness of influenza vaccination is not satisfactory and multiple factors contribute to the low effectiveness, including antigen drift, season mismatch, and manufacture technique limitations.[2,38,39] Elderly individuals have both the highest burden of disease and the lowest immune responses to vaccination.[40–42] The protection rate may be as low as 30% in elderly people after vaccination and little evidence is found supporting the benefits of influenza vaccination in the elderly.[40,41] Immunosenescence, gradual deterioration of the immune system brought on by natural aging, also plays an important role in the hyporesponsiveness of influenza vaccination.[43] Poorer nutritional status and higher rates of comorbid diseases are also important reasons for the nearly inevitable weak immune responses after vaccination in the elderly.[44,45] The TIV with high doses (4× the standard dose) induced significantly higher antibody responses in elderly people, but are not widely used.[3] Supplementation with prebiotics/probiotics may provide a simple, convenient, and practical solution.[16–18,20,46,47] Besides, probiotics consumption may have beneficial effects in preventing respiratory tract infections and influenza-related illnesses.[48,49] Our study provided comprehensive evidence that prebiotic/probiotic use will enhance the HI antibody titer after influenza vaccination. In addition, the immunogenicity of influenza vaccination may be affected by the components of vaccine strains. Compared with A/H1N1 and A/H3N2 strains, poorer antigen immunogenic responses in B strain were reported in previous studies.[50–52] Our studies also showed relatively lower HI antibody titers in B strain (Table 2). However, the beneficial effects of prebiotic/probiotic supplementation were observed in all A/H1N1, A/H3N2, and B strains. A 20% (A/H1N1), 19.5% (A/H3N2), and 13.6% (B strain) increase in HI antibody titers was observed in individuals with prebiotics/probiotics use.

Table 2 The mean hemagglutination inhibition antibody titers of vaccine strains in probiotics/prebiotics and control groups

Vaccine strain	Probiotics/ prebiotics group	Control group	Mean differences (% of increase)	*P*-value
A/H1N1	42.89	35.76	7.14 (20)	0.002
A/H3N2	105.4	88.25	17.19 (19.5)	0.01
B	34.87	30.73	4.17 (13.6)	0.03

Consumption of "good bacteria" could suppress the growth of pathogenic bacteria in the gastrointestinal tract and improve the intestinal barrier function.[6,7] The use of prebiotics/probiotics in patients with bacterial diarrhea is well known.[8,53–55] Probiotics are also used to prevent necrotizing enterocolitis and sepsis in preterm neonates and may also contribute to adjuvant therapy in eradication of *Helicobacter pylori*.[54,56–60] In addition to the beneficial effects in the gastrointestinal tract, systemic immunomodulatory effects, toll-like receptor-mediated pathways, regulatory T cell induction, natural killer cells, soluble proteins, and various cytokines were involved in the probiotic immune regulatory mechanism.[5,9–12,61] Therefore, manipulation of the

Study or subgroup	Probiotics/prebiotics Events	Total	Placebo Events	Total	Weight (%)	Odds ratio M–H, random, 95% CI	Year
Maruyama et al[26]	0	21	0	21		Not estimable	
Bunout et al[73]	1	20	2	23	6.5	0.55 (0.05, 6.59)	
Akatsu et al (paper)[30]	0	23	0	22		Not estimable	
Rizzardini et al (*Lactobacillus casei* drink)[70]	0	56	0	54		Not estimable	
Rizzardini et al (BB 12 capsule)[70]	0	53	0	48		Not estimable	
Olivares et al[67]	0	25	0	25		Not estimable	2007
Boge et al (pilot)[34]	5	44	5	42	22.9	0.95 (0.25, 3.55)	2009
Boge et al (confirmatory)[34]	14	113	16	109	66.9	0.82 (0.38, 1.78)	2009
Davidson et al[32]	0	19	1	20	3.7	0.33 (0.01, 8.70)	2011
Nagafuchi et al[28]	0	12	0	12		Not estimable	2015
Total (95% CI)		**386**		**376**	**100**	**0.80 (0.43, 1.50)**	
Total events	20		24				

Heterogeneity: τ^2=0.00; χ^2=0.43, *df*=3 (*P*=0.93); I^2=0%
Test for overall effect: *Z*=0.69 (*P*=0.49)

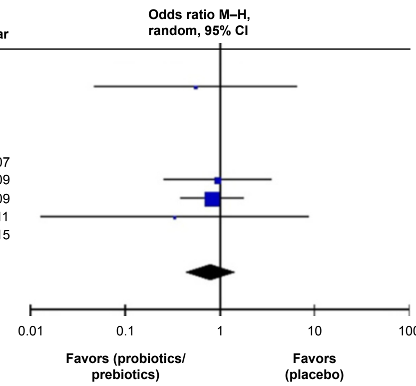

Figure 5 Forest plot of the incidence of adverse effect between prebiotic or probiotic group, and placebo group.
Abbreviation: M–H, Mantel–Haenszel method.

gut microbiota may benefit patients with systemic diseases, such as allergic diseases.[14] Reduced risks of subsequent cardiovascular diseases and metabolic outcomes were also observed.[15,62] In a report published in 2016, it was stated that probiotic-modulated gut microbiota may suppress hepatocellular carcinoma growth in mice via regulation of T cell and pro-inflammatory cytokines.[13] The use of prebiotics/probiotics/synbiotics may hold great promise for preventing and treating many extraintestinal diseases.

Probiotics are “live” bacteria, which help human to fight against pathogenic bacteria. Although the benefits of probiotics in preterm neonates are well documented, safety of probiotics in immunocompromised individuals remains a major concern.[56–58] Bacteremia caused by probiotics strains was reported in some immunocompromised patients.[63–66] Elderly people are at increased risk of being immunocompromised and the issue of safety remains important. In our meta-analysis studies, more than half of the participants were bedridden, fed with nasogastric tubes, or nursing home residents; no documented probiotics-related sepsis was reported.[26–28,30,31,34,35] Furthermore, in the subgroup analysis of our study, prebiotics were also beneficial for enhancing immune responses after influenza vaccination. Prebiotic use may be a reasonable choice for immunocompromised patients at increased risk for infection.

Our study had some limitations. First, the study design, study participants, and study period were highly heterogeneous. Further large-scale studies are warranted to confirm our findings. Second, the strain, doses, and the duration of prebiotics/probiotics supplementation differed among studies. The immune responses may vary in different supplement protocol. Further studies are required to investigate the optimal strain, dosage, and duration of probiotic consumption. Finally, the components of the influenza vaccine and prevalent influenza strains were different each year. It may be more valuable to explore the effects of probiotics with the same influenza vaccine.

Conclusion

Our study suggests that concomitant prebiotics/probiotics use might be an effective intervention to enhance the HI antibody titer following influenza vaccination (13.6%–20% increases in HI antibody titers). Adjuvant prebiotics/probiotics use may hold great promise for the improvement of immune responses following influenza vaccination. However, high heterogeneity was observed and further studies are warranted to elucidate the effectiveness and decide the optimal strains, dose, timing, and duration of supplementation.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Box S1 Detailed search strategy of systematic review

PubMed
((((Flu Vaccine* OR Afluria OR Influenza Vaccine* OR Afluria OR Influenzavirus Vaccine* OR LAIV vaccine OR FluMist OR CAIV-T vaccine OR Trivalent Live Attenuated Influenza Vaccine OR Influenza Virus Vaccine*)) OR ((((Influenza, Human) OR (Influenza* OR flu))) in All Fields
AND
((Vaccination) OR vaccine*)))) in All Fields
AND
((((((((Probiotics) OR Bifidobacterium longum) OR Lactobacillus rhamnosus) OR (Lactic acid bacteria OR Lactobacillus acidophilus OR Lactobacillus amylovorus OR Lactobacillus Streptococcus faecalis OR L. acidophilus OR B. lactis OR Bifidobacterium OR B. bifidum OR B. longum OR Bifidobacter* OR Lactobacillus casei OR Lactobacillus paracasei OR Lactobacillus rhamnosus OR Lactobacillus GG OR Culturelle)) OR probiotic*)) OR ((Prebiotics) OR ((Prebiotic* OR Oligosaccharid*)))) OR ((Synbiotics) OR Synbiotic*)) in All Fields
Embase
Influenza Vaccines OR Flu Vaccine* OR Afluria OR Influenza Vaccines OR Flu Vaccine* OR Afluria OR Influenzavirus Vaccine* OR LAIV vaccine OR FluMist OR CAIV-T vaccine OR Trivalent Live Attenuated Influenza Vaccine OR Influenza Virus Vaccine*
AND
Vaccination OR vaccine*
AND
((((((((Probiotics) OR Bifidobacterium longum) OR Lactobacillus rhamnosus) OR (Lactic acid bacteria OR Lactobacillus acidophilus OR Lactobacillus amylovorus OR Lactobacillus Streptococcus faecalis OR L. acidophilus OR B. lactis OR Bifidobacterium OR B. bifidum OR B. longum OR Bifidobacter* OR Lactobacillus casei OR Lactobacillus paracasei OR Lactobacillus rhamnosus OR Lactobacillus GG OR Culturelle)) OR probiotic*)) OR ((Prebiotics) OR ((Prebiotic* OR Oligosaccharid*)))) OR ((Synbiotics) OR Synbiotic*))
Cochrane
Influenza Vaccines OR Flu Vaccine* OR Afluria OR Influenza Vaccines OR Flu Vaccine* OR Afluria OR Influenzavirus Vaccine* OR LAIV vaccine OR FluMist OR CAIV-T vaccine OR Trivalent Live Attenuated Influenza Vaccine OR Influenza Virus Vaccine*
AND
Vaccination OR vaccine*
AND
((((((((Probiotics) OR Bifidobacterium longum) OR Lactobacillus rhamnosus) OR (Lactic acid bacteria OR Lactobacillus acidophilus OR Lactobacillus amylovorus OR Lactobacillus Streptococcus faecalis OR L. acidophilus OR B. lactis OR Bifidobacterium OR B. bifidum OR B. longum OR Bifidobacter* OR Lactobacillus casei OR Lactobacillus paracasei OR Lactobacillus rhamnosus OR Lactobacillus GG OR Culturelle)) OR probiotic*)) OR ((Prebiotics) OR ((Prebiotic* OR Oligosaccharid*)))) OR ((Synbiotics) OR Synbiotic*))
CINAHL
((((Flu Vaccine* OR Afluria OR Influenza Vaccine* OR Afluria OR Influenzavirus Vaccine* OR LAIV vaccine OR FluMist OR CAIV-T vaccine OR Trivalent Live Attenuated Influenza Vaccine OR Influenza Virus Vaccine*)) OR ((((Influenza, Human) OR (Influenza* OR flu)))
AND
((Vaccination) OR vaccine*))))
AND
((((((((Probiotics) OR Bifidobacterium longum) OR Lactobacillus rhamnosus) OR (Lactic acid bacteria OR Lactobacillus acidophilus OR Lactobacillus amylovorus OR Lactobacillus Streptococcus faecalis OR L. acidophilus OR B. lactis OR Bifidobacterium OR B. bifidum OR B. longum OR Bifidobacter* OR Lactobacillus casei OR Lactobacillus paracasei OR Lactobacillus rhamnosus OR Lactobacillus GG OR Culturelle)) OR probiotic*)) OR ((Prebiotics) OR ((Prebiotic* OR Oligosaccharid*)))) OR ((Synbiotics) OR Synbiotic*))

(*Continued*)

Box S1 (*Continued*)

Airiti
流感 OR 流行性感冒 OR 感冒
AND
疫苗
AND
益生菌 OR 乳酸菌 OR 龍根菌 OR 益菌生 OR 益生源 OR 合生元 OR 共生質 OR 合益菌
NTLTD
流感 + 流行性感冒 + 感冒
AND
疫苗
AND
益生菌 + 乳酸菌 + 龍根菌 + 益菌生 + 益生源 + 合生元 + 共生質 + 合益菌

Abbreviations: CAIV-T, cold-adapted influenza vaccine, trivalent; CINAHL, Cumulative Index to Nursing and Allied Health; LAIV, live attenuated influenza vaccine; NTLTD, Networked Digital Library of Theses and Dissertations.

Table S1 Quality assessment of each included study[a]

Study validity domains	Sequence generation	Allocation concealment	Blinding of participants and personnel and outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Probiotics						
Olivares[1]	Low	Unclear[b]	Unclear[b]	Low	Low	Low
French and Penny[2]	Low	Low	Low	Low	Low	Uncertain[f]
Boge et al (pilot)[3]	Low	Low	Low	High[d]	Low	Uncertain[f]
Boge et al (confirmed)[3]	Low	Low	Low	High[d]	Low	Uncertain[f]
Namba et al[4]	Low	Unclear[b]	Unclear[b]	High[d]	Low	Uncertain[f]
Davidson et al[5]	Low	Low	Low	Low	Low	Low
Van Puyenbroeck[6]	Low	Low	Low	High[d]	High[e]	Uncertain[f]
Rizzardini[7]	Low	Low	Low	Low	Low	Uncertain[f]
Bosch[8]	Unclear[b]	Unclear[b]	Low	High[d]	Low	High[g]
Akatsu et al[9,a] (letter)	Unclear[b]	Unclear[b]	High[c]	Low	Uncertain[b]	Uncertain[f]
Akatsu et al[10,b] (paper)	Low	Unclear[b]	Unclear[b]	Low	Low	Uncertain[f]
Jespersen[11]	Low	Low	Low	Low	High[e]	Uncertain[f]
Maruyama et al[12]	Low	Low	Low	Low	Low	Low
Prebiotics						
Bunout[13]	Low	Unclear[b]	Low	High[d]	Low	Uncertain[f]
Langkamp-Henken et al[14]	Low	Low	Unclear[b]	High[d]	Low	Uncertain[f]
Langkamp-Henken et al[15]	Unclear[b]	Low	Low	High[d]	Low	High[g]
Nagafuchi et al[16]	Unclear[b]	Unclear[b]	High[c]	Low	Low	Uncertain[f]
Lomax et al[17]	Unclear[b]	Low	Unclear[b]	High[d]	High[e]	Uncertain[f]
Akatsu et al[18]	Unclear[b]	Unclear[b]	High[c]	Low	Low	Uncertain[f]
Synbiotics						
Enami[19]	Unclear[b]	Low	Low	High[d]	Unclear	Unclear[f]

Notes: [a]Each domain has been evaluated as being "High", "Low", or "Unclear" regarding the risk of bias following the guidelines of Cochrane Collaboration's tool for assessing risk of bias. "Low" in all domains would place a study at "Low Risk of Bias"; "High" in any of the domains would place a study at "High Risk of Bias"; "Unclear" in any of the domains would place the study at "Unclear Risk of Bias". [b]Not mentioned. [c]Un-blinded, open-labeled. [d]Drop-off rate $>$10%. [e]Missing data/data lost. [f]Conflict of interest, financial supports. [g]Authors employed by funding companies.

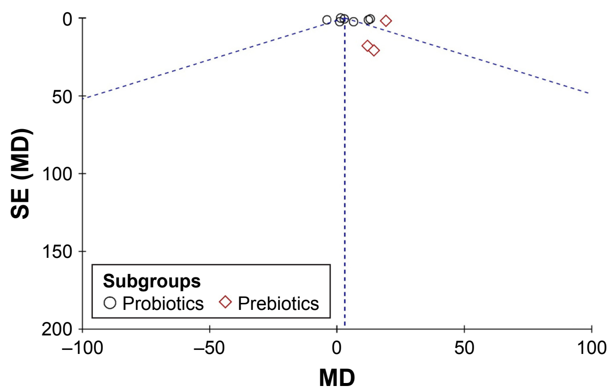

Figure S1 Funnel plot of strain A/H1N1.
Abbreviations: MD, mean difference; SE, standard error.

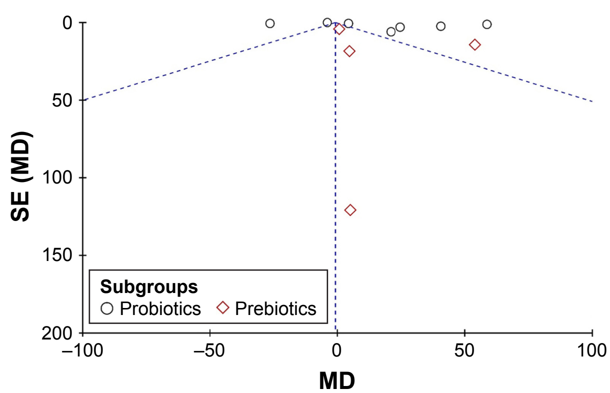

Figure S2 Funnel plot of strain A/H3N2.
Abbreviations: MD, mean difference; SE, standard error.

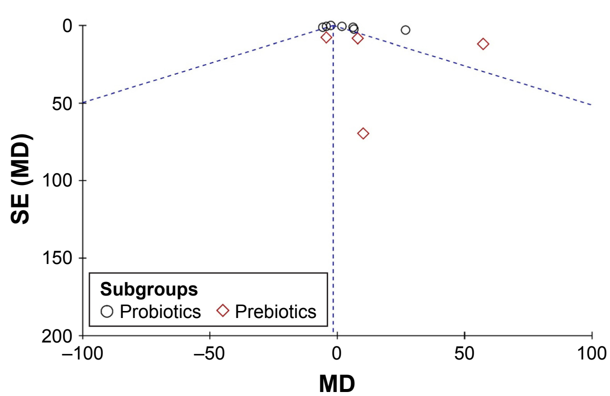

Figure S3 Funnel plot of strain B.
Abbreviations: MD, mean difference; SE, standard error.

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