Probiotics for the Prevention of Ventilator-Associated Pneumonia: A Meta-Analysis of Randomized Controlled Trials

Minmin Su, Ying Jia, Yan Li, Dianyou Zhou, and Jinsheng Jia

BACKGROUND: Ventilator-associated pneumonia (VAP) is a common and serious complication of mechanical ventilation. We conducted a meta-analysis of published randomized controlled trials to evaluate the efficacy and safety of probiotics for VAP prevention in patients who received mechanical ventilation. METHODS: We searched a number of medical literature databases to identify randomized controlled trials that compared probiotics with controls for VAP prevention. The results were expressed as odds ratios (OR) or mean differences with accompanying 95% CIs. Study-level data were pooled by using a random-effects model. Data syntheses were accomplished by using statistical software. RESULTS: Fourteen studies that involved 1,975 subjects met our inclusion criteria. Probiotic administration was associated with a reduction in VAP incidence among all 13 studies included in the meta-analysis (OR 0.62, 95% CI 0.45–0.85; $P = .003$; $I^2 = 43\%$) but not among the 6 double-blinded studies (OR 0.72, 95% CI 0.44–1.19; $P = .20$; $I^2 = 55\%$). We found a shorter duration of antibiotic use for VAP (mean difference −1.44, 95% CI −2.88 to −0.01; $P = .048$, $I^2 = 30\%$) in the probiotics group than in the control group, and the finding comes from just 2 studies. No statistically significant differences were found between the groups in terms of ICU mortality (OR 0.95, 95% CI 0.67–1.34; $P = .77$; $I^2 = 0\%$), ICU stay (mean difference –0.77, 95% CI –2.58 to 1.04; $P = .40$; $I^2 = 43\%$), duration of mechanical ventilation (mean difference –0.91, 95% CI –2.20 to 0.38; $P = .17$; $I^2 = 25\%$), or occurrence of diarrhea (OR 0.72, 95% CI 0.45–1.15; $P = .17$; $I^2 = 41\%$). CONCLUSIONS: The meta-analysis results indicated that the administration of probiotics significantly reduced the incidence of VAP. Furthermore, our findings need to be verified in large-scale, well-designed, randomized, multicenter trials. *Key words: ventilator-associated pneumonia; critical care; probiotics; prevent; randomized controlled trial; meta-analysis.* [Respir Care 2020;65(5):673–685. © 2020 Daedalus Enterprises]

Introduction

Ventilator-associated pneumonia (VAP) is defined as an infectious inflammatory reaction of the lung parenchyma that occurs after mechanical ventilation for >48 h. VAP is a common and severe complication in mechanically ventilated patients and can lead to a prolonged hospital stay, increased medical costs, and a higher mortality risk.[1,2] The incidence of VAP in the United States is ~4.4 cases per 1,000 mechanical ventilation days,[3] and it has become an important cause of death in patients who receive mechanical ventilation. The pathogenesis of VAP is mainly due to bacterial colonization of the upper respiratory tract and inhalation of contaminated secretions into the lower respiratory tract.[4] Although antibiotics can effectively reduce the VAP incidence by eliminating pathogenic bacteria,[4] antibiotic abuse can lead to an increase in drug resistance. Consequently, finding new safe and effective preventive measures is important.[5]

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Recently, the intestinal microflora has attracted great attention from researchers. Results of studies indicate that intestinal microecological imbalances are associated with the development of obesity, diabetes, and inflammatory diseases.[6-8] Probiotics are defined as active microorganisms that can produce positive effects in the host when administered at the appropriate dosage.[6] They can selectively stimulate the growth of some bacteria and improve the microecological balance of the host.[9] The positive effects of probiotics on VAP may include (1) strengthening the gut barrier function, (2) reducing the overgrowth of potential pathogens, and (3) stimulating immune responses.[10,11]

However, whether probiotics can effectively prevent VAP remains controversial. Currently, results of 6 randomized controlled trials (RCTs) indicate that the administration of probiotics has a positive effect on reducing VAP incidence.[4,12-16] Results of 8 other RCTs show that probiotics had no significant effect on the prevention of VAP.[17-20,22-25] In 2017, Weng et al[26] conducted a meta-analysis and found that probiotics reduced the incidence of VAP in mechanically ventilated subjects. There may be great population heterogeneity in the meta-analysis by Weng et al[26] because it included two articles that involved pediatric and neonatal subjects. In the 2 years since the meta-analysis by Weng et al,[26] 3 RCTs have been published.[15,17,18] To elucidate the latest and most convincing evidence, we carried out a meta-analysis of the published RCTs to evaluate the effects of probiotics on VAP prevention in subjects on mechanical ventilation.

QUICK LOOK

Current knowledge

Ventilator-associated pneumonia (VAP) is a common complication in mechanically ventilated patients. Many studies have evaluated the role of probiotics in the prevention of VAP, but there is no clear conclusion.

What this paper contributes to our knowledge

Our meta-analysis indicated that probiotics can effectively reduce the incidence of VAP. Additional large-scale and multi-center randomized controlled trials are needed to further verify the role of probiotics in the prevention of VAP.

Methods

Search Strategy

Two of us (MS, YJ) systematically retrieved studies from PubMed, EMBASE, and Cochrane databases (each database was searched on February 28, 2019). The search phrases used included "probiotics" or "probiotic" or "synbiotics" or "synbiotic" or "*Lactobacillus*" or "lactobacilli" or "*Bifidobacterium*" and "pneumonia, ventilator-associated" or "pneumonia, ventilator associated" or "ventilator-associated pneumonia" or "VAP" or "nosocomial pneumonia" or "hospital acquired pneumonia" or "respiratory infection" or "critically ill" and "randomized, controlled trial" or "randomized" or "placebo." Our search strategy for PubMed is provided in the supplementary materials (see the supplementary materials at http://www.rcjournal.com).

Study Selection and Outcome Assessment

Eligibility Criteria. We included studies that met the following criteria: (1) involved adults receiving mechanical ventilation, (2) compared probiotics with a placebo or standard therapy, (3) assessed VAP as the outcome, and (4) were RCTs (including multi-arm random clinical studies).

Exclusion Criteria. We excluded studies that met the following criteria: (1) included only interventions with prebiotics and without any additional probiotics, and (2) analyzed the same cohort as other studies (we selected the one with the largest sample size or the longest follow-up time).

Outcome Assessment. The primary outcome was VAP incidence, and the secondary outcomes were ICU mortality, length of ICU stay, duration of mechanical ventilation, antibiotic use for VAP, and diarrhea. All the studies were screened independently by MS and YJ. After removing duplicate results, we skimmed through the topics and abstracts to preliminarily determine the eligible studies. We then evaluated the full texts to determine the studies that were ultimately included in the meta-analysis. Disagreements were resolved by discussion or by a third reviewer (YL).

Data Extraction

MS and YJ independently extracted data by using a Microsoft Excel spreadsheet (XP Professional Edition, Microsoft, Redmond, Washington); the extracted data included the study characteristics (the first author, publication year, design, duration, participants, interventions, definition of VAP) and the outcomes. Missing means and SDs were estimated by the methods of Luo et al[19] and Wan et al,[20] respectively. For multiple treatment arm studies, we pooled the data by using the methods described in the Cochrane Handbook.[21]

Quality Assessment

The bias of the studies was assessed as described in the Cochrane Handbook[21] and included characteristics such as random sequence generation, allocation concealment, blinding of patients, blinding of outcome assessments, completeness of outcome data, selective reporting, and other biases. Differences were resolved by discussion or by a third reviewer (YL).

Data Pooling and Analysis

We calculated the odds ratios (OR) with 95% CIs for binary variables and mean differences with 95% CIs for continuous variables. Heterogeneity among the studies was evaluated by the I^2 statistics. Studies with $I^2 > 50\%$ were considered to have significant heterogeneity. The study-level data were pooled by using a random-effects model. Data analysis and synthesis were accomplished by using RevMan 5.3 (Cochrane Collaboration, Oxford, United Kingdom) and Stata 14.0 (Stata, College Station, Texas). Publication bias was also estimated by using a contour-enhanced funnel plot. We performed subgroup analyses for the different species of probiotics, diagnostic criteria for VAP, types of trial design. and pathogens. Sensitivity analyses were conducted by excluding studies with a high risk of bias and inexplicit diagnostic criteria to evaluate the stability of the primary outcome. In addition, a sensitivity analysis was performed to identify the source of heterogeneity among the studies.

Results

Included Studies

We retrieved a total of 413 articles from the above databases. A total of 283 articles remained after deleting duplicate records; 262 were excluded based on their titles and abstracts. After reading the full texts, 14 RCTs were included in the analysis. The study screening process is shown in Figure 1.

Characteristics of the Included Studies

The characteristics of all of the RCTs included in this meta-analysis are shown in Table 1.[4,12-18,22-25,27,28] Most of the included studies were published within the past 10 years. The sample sizes ranged from 35 to 300 (median, 150). Thirteen studies reported the VAP incidence, 6 reported ICU mortality, 10 reported ICU stays, 8 reported the duration of mechanical ventilation, 2 reported antibiotic use for VAP, and 6 reported the occurrence of diarrhea. Five studies used a single probiotic (*Lactobacillus rhamnosus,*[14,23] *Lactobacillus plantarum* 299,[17,25] or *Lactobacillus casei*[26]), 5 studies used multiple probiotics,[4,15,18,22,28] and 4 studies used a synbiotic formula (Synbiotic 2000Forte Medipharm, Kågeröd, Sweden and Des Moines, IA).[12,13,16,24] The studies included 7 double-blind trials,[12-14,18,22-24] 3 single-blind trials,[15,16,28] and 4 open trials.[4,17,25,27] With regard to the diagnostic criteria for VAP, 8 studies required microbiologic confirmation,[13,17,18,22,23,25,27,28] 4 studies used only clinical criteria,[4,12,14,24] and 2 studies did not provide specific diagnostic criteria.[15,16] The outcome data extracted from the RCTs included in the meta-analysis are shown in Table 2.

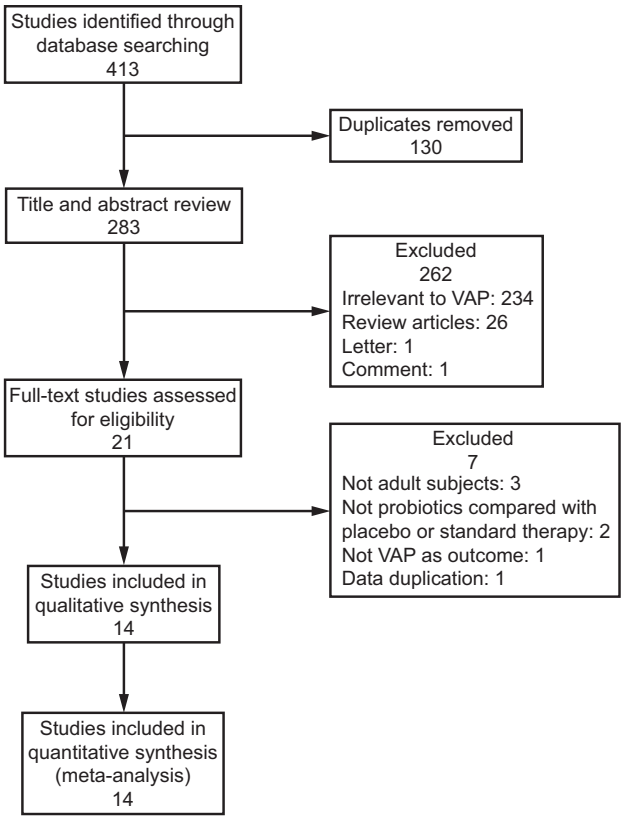

Fig. 1. Flow chart.

Risk of Bias in the Included Studies

The risk of bias in the included studies is shown in Figures 2 and 3. Five studies were considered to have a high risk bias[4,14,17,25,27] for the following reasons: the trials were open studies and did not perform blinding of participants and personnel,[4,17,25,27] there were no blind evaluations of the outcome,[25,27] and the reasons for the lack of data among the groups were not similar.[4,14] In addition, 7 studies[14-16,22-24,28] reported random sequence generation, 6 studies[15,16,22-24,28] reported allocation concealment, and all of the studies were estimated to have a low risk of reporting bias.

Table 1. Features of the Included Studies

Study, y	Design/Duration	Participants	Intervention	Definition of VAP
Barraud et al,[22] 2010	Double blind/90 d	Adults intubated and on MV ≥ 2 d; $N = 167$	Probiotic: multispecies probiotics*/control: placebo; doses: 2×10^{10} CFU/d; admin: enteral feeding tube	1. CRI + 1 sign: (1) PTS, (2) T ≥ 38.3°C, (3) WBC ≥ 10,000/mm^3; 2. Positive quantitative cultures from BAL
Forestier et al,[23] 2008	Double blind/not stated	Age ≥ 18 y; stay > 48 h and an NT; $N = 236$ (relevant $n = 202$)	Probiotic: *L. casei rhamnosus*/control: placebo; doses: 2×10^9 CFU/d; admin: NT or oral	CRI + 1 positive sample† + 1 sign: (1) PTS, (2) T > 38.5°C, (3) positive blood culture, (4) mini-BAL with >5% of cells with intracellular bacteria
Giamarellos-Bourboulis et al,[12] 2009	Double blind/28 d	Multiple organ injuries; tracheal intubation; mechanically ventilated in the ICU; $N = 72$	Probiotic: Synbiotic 2000Forte‡/control: placebo; doses: 10^{11} CFU/d; admin: NT or gastrostomy	All of the following: (1) new or persistent consolidation on a lung radiograph, (2) PTS, (3) CPIS > 6
Klarin et al,[17] 2018	Open label/6 mo	Age ≥ 18 y, critically ill and ventilated ≥ 24 h; $N = 150$	Probiotic: *L. plantarum* 299/control: standard protocol§; doses: 10^{10} CFU/d; admin: applied to the mucosal surface of the oral cavity	CRI + 3 sign: (1) PTS, (2) positive culture of tracheal aspirates after MV 48 h, (3) T (rectal or urine bladder) > 38.0°C or < 35.5 °C, (4) WBC > 12,000/mm^3 or < 3,000/mm^3, or a rapid increase
Knight et al,[24] 2009	Double blind/28 d	Subjects who were critically ill and mechanically ventilated; without contraindications to enteral nutrition; ventilated ≥ 48 h; $N = 300$	Probiotic: Synbiotic 2000Forte‡/control: placebo; doses: 2×10^{10} CFU/d; admin: NT or orogastric tube	CRI + 2 sign: (1) T > 38.0°C, (2) WBC > 12,000/mm^3 or < 4,000/mm^3, (3) PTS
Kotzampassi et al,[13] 2006	Double blind/15 d	Organ-system traumas ≥ 2; mechanically ventilated; age > 18 y; life expectancy > 15 d; $N = 77$	Probiotic: Synbiotic 2000Forte‡/control: placebo; doses: 10^{11} CFU/d; admin: NT or gastrostomy	All of the following: (1) CRI, (2) PTS, (3) T > 38.5°C, (4) WBC > 12,000/mm^3 or < 4,000/mm^3, (5) positive quantitative cultures from BAL
Mahmoodpoor et al,[18] 2019	Double blind/2 wk	Subjects who were critically ill, age >18 y; admitted to the ICU; mechanically ventilated > 48 h; $N = 120$	Probiotic: probiotic preparation‖/control: placebo; doses: 2×10^{10} CFU/d; admin: feeding tube	CRI + 2 sign: (1) T > 38.0°C or < 36.0°C, (2) leukocytosis or leucopenia, (3) purulent sputum underwent BAL
Morrow et al,[14] 2010	Double blind/not stated	Age ≥ 19 y; expected invasive ventilated ≥ 72 h; $N = 146$	Probiotic: *L. rhamnosus* GG/control: placebo; doses: 4×10^9 CFU/d; admin: NT or oropharynx tube	CRI + 2 sign: (1) T > 38.5°C or < 35.0°C, (2) WBC > 10,000/mm^3 or < 3,000/mm^3, (3) PTS
Oudhuis et al,[25] 2011	Open label/28 d	Age ≥ 18 y; expected mechanically ventilated ≥ 48 h (254 [relevant $n = 248$])	Probiotic: *L. plantarum* 299, 299v/control: SDD; doses: 10^{10} CFU/d; admin: NT	Confirmation of clinically suspected VAP required ≥ 2% cells that contained intracellular organisms and/or a quantitative culture result of C10^4 CFU/mL in BAL fluid
Rongrungruang,[27] 2015	Open label/90 d	Adult subjects; expected mechanical ventilation ≥ 72 h; no VAP at enrollment; $N = 150$	Probiotic: *L. casei*/control: 2% chlorhexidine solution 4 times a day; doses: 10^{10} CFU/d; admin: oral care and enteral feeding	CRI + 3 sign: (1) T > 38.0°C or < 35.5°C, (2) WBC > 10,000/mm^3 or < 3,000/mm^3, (3) PTS, (4) positive semiquantitative culture of tracheal aspirate samples

(Continued)

Table 1. Continued

Study, y	Design/Duration	Participants	Intervention	Definition of VAP
Shimizu et al,[15] 2018	Single blind/4 wk	Age ≥ 16 y; mechanically ventilated within 3 d after admission to the ICU; diagnosed sepsis; $N = 77$	Probiotic: Yakult BL Seichoyaku (Yakult Honsha, Tokyo, Japan)¶/control: no additional products; doses: 6×10^8 CFU/d; admin: nasal tube	Arises >48–72 h after endotracheal intubation
Spindler-Vesel et al,[16] 2007	Single blind, multiple arms/not stated	Multiple injuries; ICU stay ≥ 4 d; ISS = 18; $N = 113$	Probiotic: Synbiotic 2000Forte‡/control: 3 arms**; doses: 10^{10} CFU/d; admin: intragastric tube	No definition
Tan et al,[28] 2011	Single blind/28 d	Closed head injury only; admission within 24 h after trauma; Glasgow Coma Scale score = 5 ~ 8; age 18–60 y; fed via NT within 48 h after admission; $N = 52$ (relevant $n = 35$)	Probiotic: Golden Bifid (Shuangqi Pharmaceutical, Inner Mongolia, China)††/control: enteral nutrition; doses: 4.2×10^8 CFU/d; admin: NT	CRI + 2 sign: (1) T > 38.0°C or < 35.5°C, (2) WBC > 12,000/mm^3 or < 4,000/mm^3, (3) PTS, (4) positive semiquantitative cultures of TBS
Zeng et al,[4] 2016	Open label/14 d	Critically ill; ages ≥18 y; expected mechanical ventilation ≥ 48 h; $N = 250$	Probiotic: probiotics capsules‡‡/control: standard strategies§§; doses: 1.5×10^{10} CFU/d; admin: NT	CRI ≥ 48 h + 2 sign: (1) T > 38.0°C or < 35.5°C, (2) WBC > 12,000/mm^3 or < 3,000/mm^3 or left shift, (3) TBS

* Mainly *L. rhamnosus* GG, but also *L. casei, Lactobacillus acidophilus,* and *Bifidobacterium bifidum.*

† Protected specimen brush or plugged telescoping catheter for broncho-alveolar minilavage [$>10^3$ colony-forming units (CFUs)/ml] or endotracheal aspirate with [$>10^5$ CFUs/ml and >25 leuco cytes/high-power field]).

‡ A combination of 10^{11} CFU of each one of the following LAB (lactic acid bacteria): *Pediacoccus pentoseceus, Leuconostoc mesenteroides, Lactobacillus paracasei*and *Lactobacillus plantarum* containing also insulin, betaglucan, pectin, and resistant starch as bioactive fibers.

§ Prostheses were removed; secretions were removed by suction; teeth were brushed by using toothpaste, and all mucosal surfaces were cleansed with swabs that had been moistened with a 1 mg/mL chlorhexidine solution.

‖ Each capsule contained 10^{10} bacteria that consisted of Lactobacillus species (L. casei, L. acidophilus, L. rhamnosus, L. bulgaricus), Bifidobacterium species (B. breve, B. longum), and Streptococcus thermophilus.

¶ Contained 1×10^8 living bacteria of the *B. breve* strain Yakult/g and 1×10^8 living bacteria of the *L. casei* strain Shirota/g.

** Alitraq (Abbott-Ross, Abbott Park, IL) (5.25 g protein, 16.5 g carbohydrate, 1.55 g fat, 1.55 g glutamine, 446 mg arginine, and 154 mg α-linolenic acid per 100 mL), Nova Source (Novartis Medical Nutrition, Basel, Switzerland) (4.1 g protein, 14.4 g carbohydrate, 3.5 g fat, and 2.2 g fermentable fibers as fermentable guar gum per 10 mL); Nutricomp peptide (B. Braun, Melsungen, Germany) (4.5 g hydrolyzed protein, 16.8 g carbohydrate, and 1.7 g fat per 100 mL).

†† Each sachet of probiotics contained 0.5×10^8 *B. longum*, 0.5×10^7 *L. bulgaricus* and 0.5×10^7 *Streptococcus thermophilus.*

‡‡ Each probiotics capsule contained active *Bacillus subtilis* and *Enterococcus faecalis* at a concentration of 4.5×10^9 per 0.25 g and 0.5×10^9 per 0.25 g, respectively.

§§ Daily screening for weaning potential and weaning from mechanical ventilation as soon as possible, hand hygiene, aspiration precautions, and prevention of contamination (the "WHAP" strategies).

VAP = ventilator-associated pneumonia
MV = mechanical ventilation
Probiotic = probiotic group
Control = controlled group
Admin = administration
CRI = new, progressive, or persistent infiltration on chest radiograph
PTS = purulent tracheal (tracheobronchial) secretion
T = temperature
WBC = white blood cell count
BAL = bronchoalveolar lavage
NT = nasogastric tube
L. casei rhamnosus = *Lactobacillus casei rhamnosus*
CPIS = clinical pulmonary infection score
L. plantarum = *Lactobacillus plantarum*
L. rhamnosus GG = *Lactobacillus rhamnosus* GG
SDD = selective decontamination of the digestive tract
L. casei = *Lactobacillus casei*
ISS = injury severity score
TBS = tracheobronchial secretions

Table 2. Outcome Data Extracted from the RCTs included in the Meta-Analysis

Study: Group	VAP Incidence (*n*/*N*)	ICU Mortality (*n*/*N*)	Length of ICU Stay (mean + SD d)	Duration of Mechanical Ventilation (mean + SD d)	Antibiotic Use for VA (mean + SD d)	Occurrence of Diarrhea, (*n*/*N*)	Etiology (*n*/*N*)						
							Acinetobacter baumannii	Entero-bacteriaceae	*Haemophilus influenzae*	*Klebsiella pneumoniae*	MRSA	*Pseudomonas aeruginosa*	*Stenotrophomonas maltophilia*
Barraud et al,[22] 2010													
Probiotic	23/78	21/78	18.7 ± 12.4	NA	NA	48/78	NA	NA	NA	NA	NA	NA	NA
Control	15/71	21/71	20.2 ± 20.8	NA	NA	42/71	NA	NA	NA	NA	NA	NA	NA
Forestier et al,[23] 2008													
Probiotic	24/99	NA	NA	NA	NA	NA	NA	9/99	NA	NA	NA	3/99	NA
Control	24/103	NA	NA	NA	NA	NA	NA	5/103	NA	NA	NA	8/103	NA
Giamarellos-Bourboulis et al,[12] 2009													
Probiotic	15/36	NA	NA	NA	NA	NA	5/36	NA	NA	5/36	NA	3/36	NA
Control	16/36	NA	NA	NA	NA	NA	12/36	NA	NA	2/36	NA	2/36	NA
Klarin et al,[17] 2018													
Probiotic	7/69	10/69	7.6 ± 6.0	10.2 ± 18.3	NA	NA	NA	NA	NA	NA	NA	NA	NA
Control	10/68	11/68	6.5 ± 5.0	9.3 ± 16.1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Knight et al,[24] 2009													
Probiotic	12/130	28/130	6.7 ± 6.0	5.4 ± 5.2	NA	7/130	3/130	6/130	0/130	NA	0/130	0/130	0/130
Control	17/129	34/129	8.1 ± 8.2	6.4 ± 6.0	NA	9/129	1/129	7/129	1/129	NA	1/129	1/129	1/129
Kotzampassi et al,[13] 2006													
Probiotic	19/35	NA	27.7 ± 15.2	16.7 ± 9.5	NA	5/35	NA	NA	NA	NA	NA	NA	NA
Control	24/30	NA	41.3 ± 20.5	29.7 ± 16.5	NA	10/30	NA	NA	NA	NA	NA	NA	NA
Mahmoodpoor et al,[18] 2019													
Probiotic	NA	5/48	14.2 ± 8.6	8.8 ± 4.8	NA	7/48	0/48	0/48	NA	1/48	NA	0/48	NA
Control	NA	6/52	21.1 ± 5.7	12.1 ± 7.1	NA	15/52	1/52	1/52	NA	2/52	NA	2/52	NA
Morrow et al,[14] 2010													
Probiotic	13/68	NA	14.8 ± 11.8	9.5 ± 6.3	5.6 ± 7.8	46/68	3/68	2/68	1/68	1/68	4/68	0/68	0/68
Control	28/70	NA	14.6 ± 11.6	9.6 ± 7.2	8.6 ± 10.3	57/70	2/70	3/70	3/70	3/70	6/70	6/70	4/70
Oudhuis et al,[25] 2011													
Probiotic	10/129	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Control	9/119	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Rongrungruang,[27] 2015													
Probiotic	18/75	NA	33.3 ± 19.6	12.8 ± 20.2	NA	19/75	8/75	3/75	NA	NA	0/75	2/75	2/75
Control	22/75	NA	18.8 ± 5.2	13.5 ± 22.5	NA	14/75	6/75	3/75	NA	NA	1/75	3/75	3/75
Shimizu et al,[15] 2018													
Probiotic	5/35	NA	26.6 ± 23.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Control	18/37	NA	30.1± 21.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Spindler-Vesel et al,[16] 2007													
Probiotic	4/26	2/26	14.1 ± 10.0	12.2 ± 8.9	NA	NA	NA	NA	NA	NA	NA	NA	NA
Control	34/87	5/87	13.5 ± 11.5	10.5 ± 7.4	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tan et al,[28] 2011													
Probiotic	7/16	NA	NA	NA	NA	NA	2/16	0/16	NA	0/16	NA	5/16	NA
Control	13/19	NA	NA	NA	NA	NA	6/19	4/19	NA	2/19	NA	9/19	NA
Zeng et al,[4] 2016													
Probiotic	43/118	15/118	21.5 ± 13.5	13.1 ± 9.8	6.8 ± 2.0	NA	10/118	3/118	NA	NA	6/118	13/118	4/118
Control	59/117	9/117	30.1 ± 33.8	19.5 ± 11.3	7.9 ± 2.9	NA	14/117	3/117	NA	NA	7/117	19/117	1/117

RCT = randomized controlled trial
VAP = ventilator-associated pneumonia
MRSA = methicillin-resistant *Staphylococcus aureus*
NA = not applicable

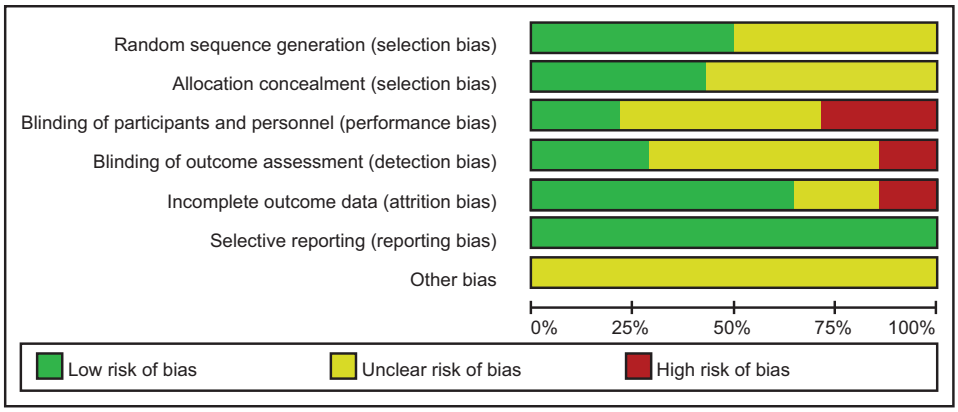

Fig. 2. Judgments about each risk of bias item.

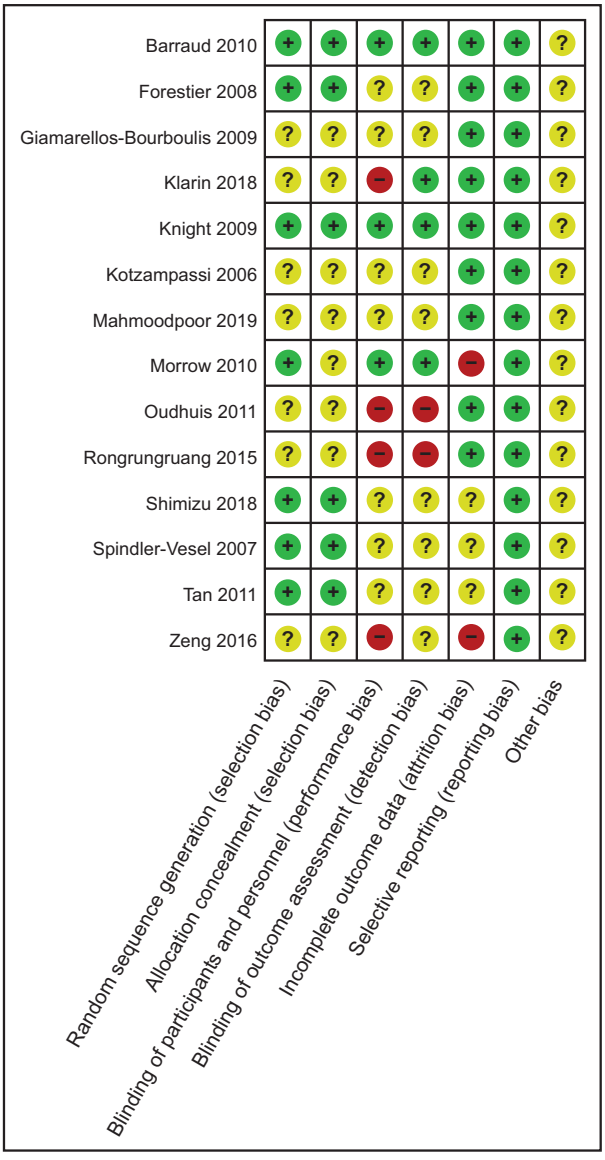

Fig. 3. Judgments about each risk of bias item for each included study.

Outcomes

We performed our meta-analysis based on a per-protocol analysis and intention-to-treat analysis, and the results were consistent. The intention-to-treat analysis is provided in the supplementary materials (see the supplementary materials at http://www.rcjournal.com).

Primary Outcome: VAP Incidence. Thirteen RCTs (1,875 subjects) reported a VAP incidence. The analysis showed that a VAP incidence in the probiotics group was significantly lower than that in the control group (OR 0.62, 95% CI 0.45-0.85; $P = .003$; $I^2 = 43\%$) (Fig. 4). After removing the studies with a high risk of bias[4,14,17,25,27] and inexplicit diagnostic criteria,[15,16] the results remained statistically significant.

Secondary Outcomes. The secondary outcomes are summarized in Table 3. No statistically significant differences were found between the groups in terms of ICU mortality (Fig. 5), ICU stay (Figs. 6 and 7), duration of mechanical ventilation (Figs. 8 and 9), or occurrence of diarrhea (Fig. 10). We found a shorter duration of antibiotic use for VAP in the probiotics group than in the control group (Fig. 11).

Subgroup Analyses

The results of subgroup analysis for the primary outcome are outlined in Table 4; further details are provided in the supplementary materials (see the supplementary materials at http://www.rcjournal.com).

Publication Bias

A contour-enhanced funnel plot (Fig. 12) revealed apparent asymmetry that suggested the presence of a potential publication bias.

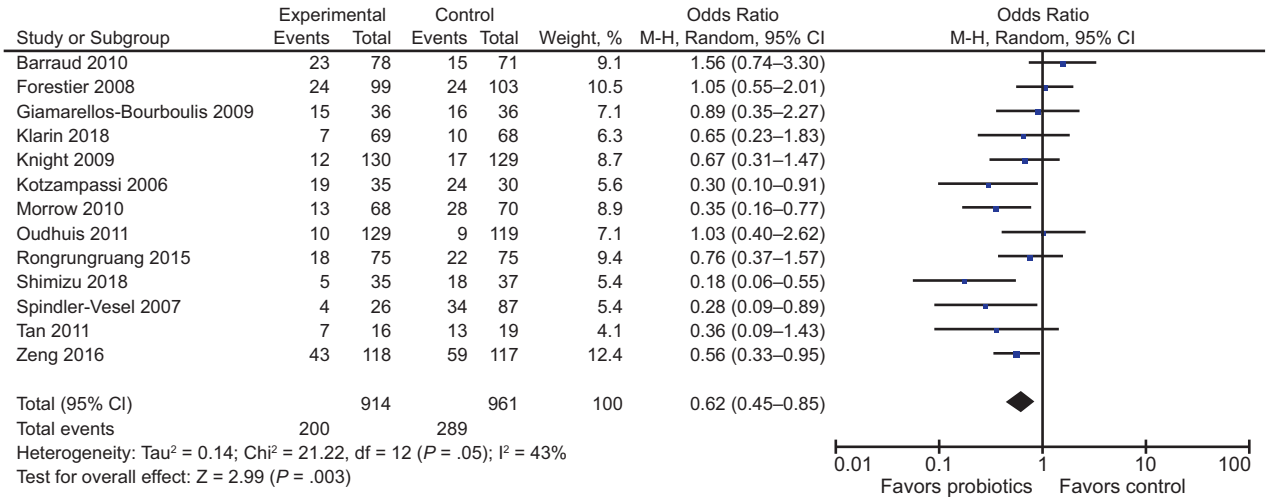

Fig. 4. A forest plot of ventilator-associated pneumonia (VAP) incidences.

Table 3. The Secondary Outcomes

Outcome or Subgroup Title	Studies, no.	Subjects, *N*	Statistical Method	Effect Size
ICU mortality	6	993	OR (M-H, random, 95% CI)	0.95 (0.67, 1.34)
Length of ICU stay, d	10	1,418	Mean difference (IV, random, 95% CI)	–1.29 (–4.74, 2.15)
Removed studies that led to high heterogeneity	7	1,103	Mean difference (IV, random, 95% CI)	–0.77 (–2.58, 1.04)
Duration of mechanical ventilation, d	8	1,197	Mean difference (IV, random, 95% CI)	–2.37 (–4.67, –0.08)
Removed studies that led to high heterogeneity	6	897	Mean difference (IV, random, 95% CI)	–0.91 (–2.20, 0.38)
Antibiotic use for VAP, d	2	373	Mean difference (IV, random, 95% CI)	–1.44 (–2.88, –0.01)
Occurrence of diarrhea	6	861	OR (M-H, random, 95% CI)	0.72 (0.45, 1.15)

OR = odds ratio
M-H = Mantel-Haenszel
IV = inverse variance
VAP = ventilator-associated pneumonia

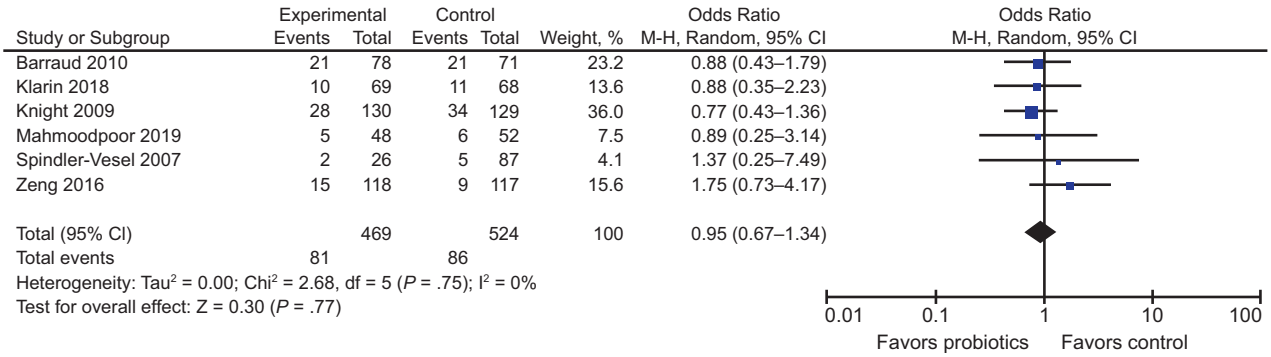

Fig. 5. A forest plot of ICU mortality.

Discussion

Our meta-analysis demonstrated that probiotics significantly decreased the incidence of VAP. No appreciable effects were conferred by probiotics on ICU mortality, length of ICU stay, duration of mechanical ventilation, or occurrence of diarrhea. We found a reduction in the use of antibiotics for VAP among the subjects treated with probiotics, but an insufficient sample limited the strength of this result. Although no adverse effects related to probiotics were found in the included studies, it is vital to conduct safety monitoring in future clinical trials.

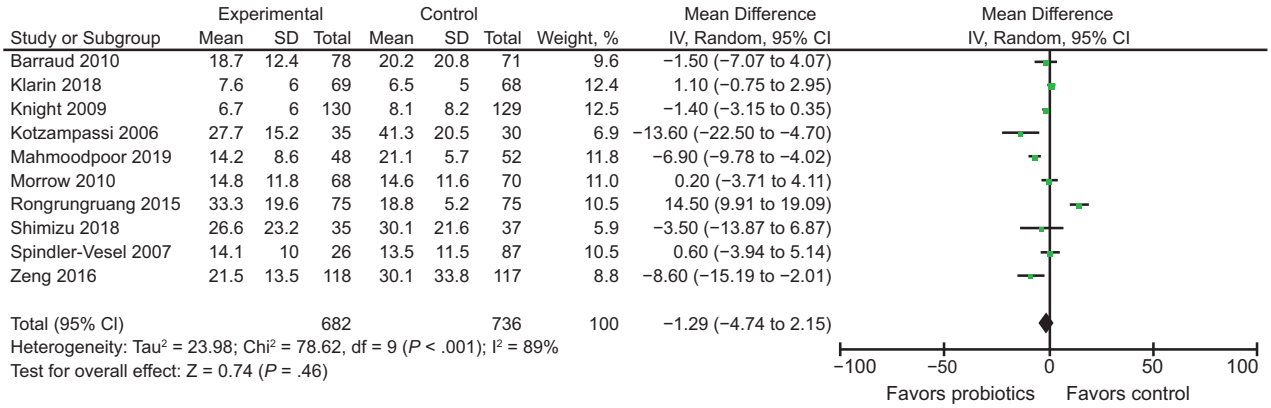

Fig. 6. A forest plot of length of ICU stay (days).

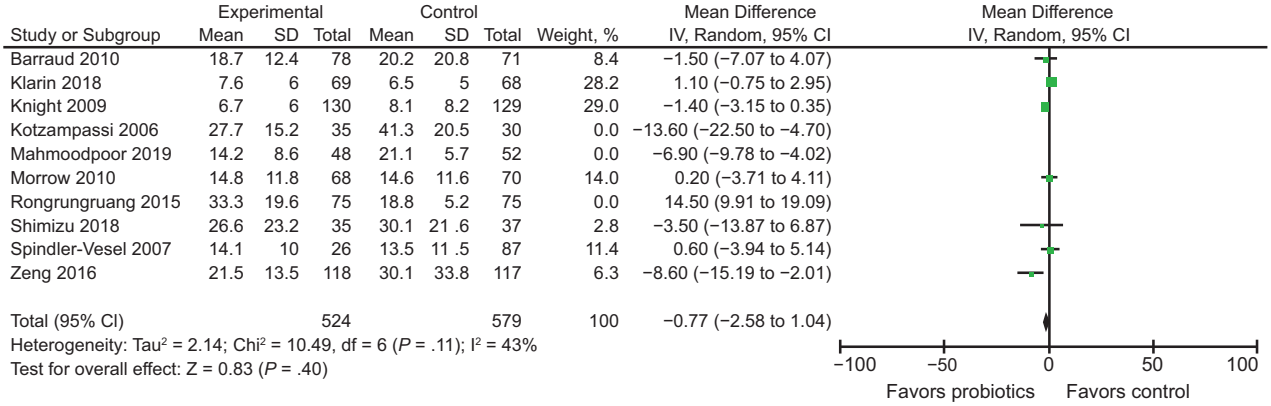

Fig. 7. A forest plot of the sensitivity analysis for length of ICU stay (days).

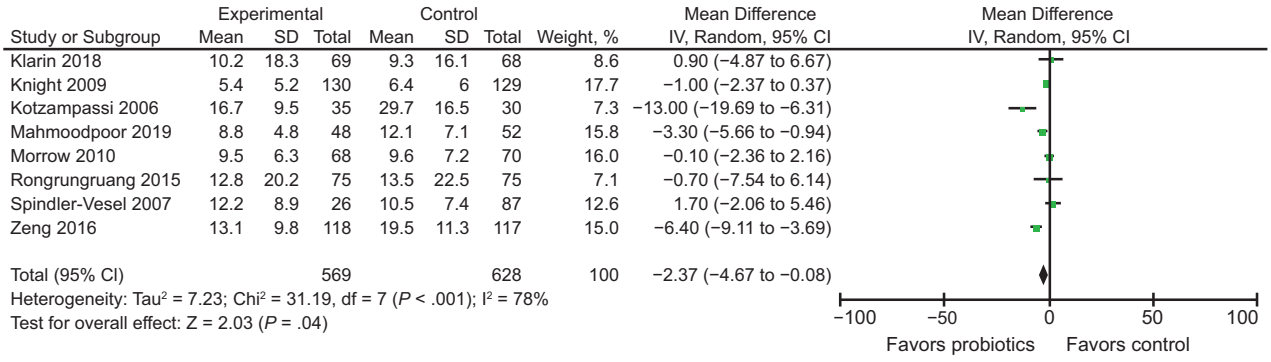

Fig. 8. A forest plot of duration of mechanical ventilation (days).

Our conclusions were consistent with 3 previously published meta-analyses (Weng et al,[26] Bo et al,[29] and Siempos et al[30]). By including more recent studies, our meta-analysis further confirmed these findings. With regard to the meta-analysis by Weng et al,[26] we did not think that two of the studies[31,32] were appropriate for inclusion because the participants in Banupriya et al[31] and Li et al[32] were children and neonate, respectively. The intestinal microenvironment of children is significantly different from that of adults. This clinical heterogeneity may have caused bias in the outcome. We excluded these 2 studies and included 3 newly published RCTs (Mahmoodpoor et al,[18] Klarin et al,[17] and Shimizu et al[15]) and one other RCT (Kotzampassi et al[13]), which made our results more convincing. The study by Kotzampassi et al[13] was also included in the studies of Bo et al[29] and Siempos et al.[30] The meta-analysis of Wang[33] suggested that probiotics had no effect on VAP prevention; however, differences in the probiotic strains and VAP

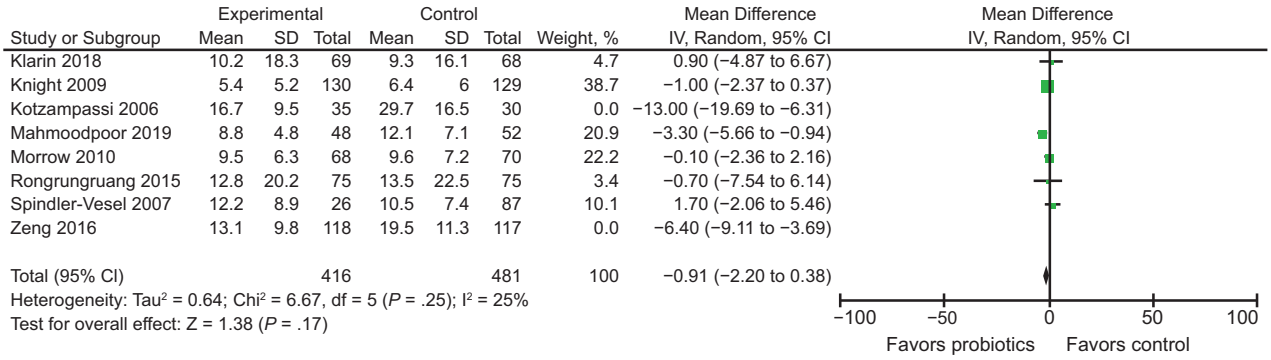

Fig. 9. A forest plot of the sensitivity analysis for duration of mechanical ventilation.

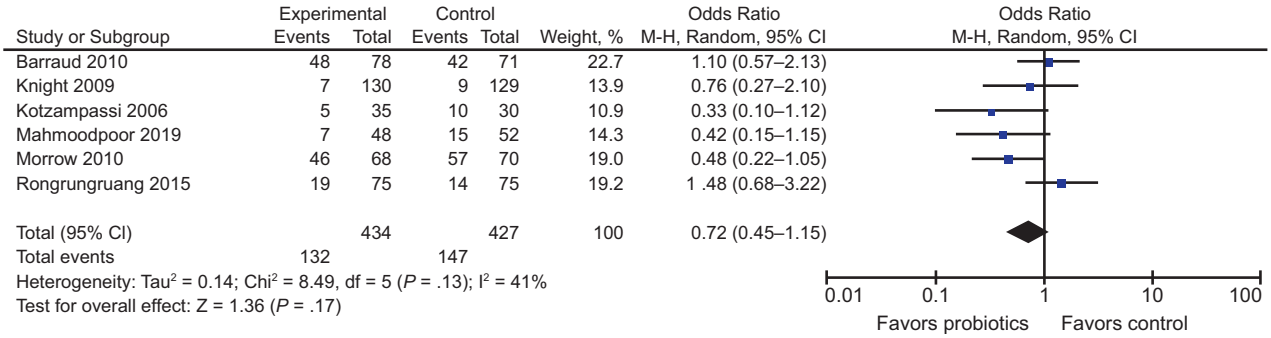

Fig. 10. A forest plot of occurrence of diarrhea.

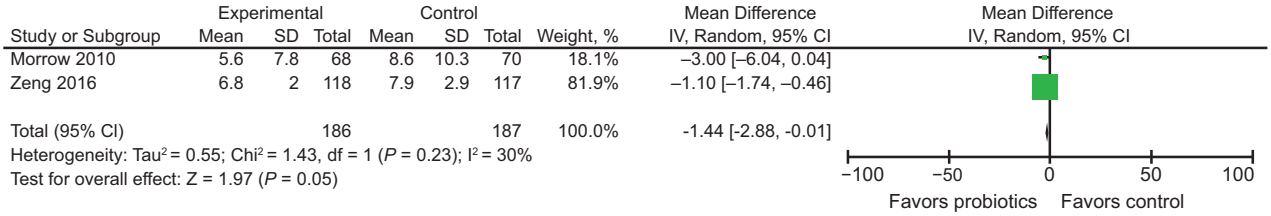

Fig. 11. A forest plot of antibiotic use for ventilator-associated pneumonia (VAP) (days).

diagnostic criteria may be the reason for the inconsistent results. An insufficient sample size greatly weakened their conclusions.

To date, measures to prevent VAP mainly include subglottic secretion drainage, the use of oral antiseptics, and a shortened duration of mechanical ventilation, but only a limited number of positive results have been achieved.[10] Analysis of a substantial number of findings suggested that probiotics were a promising option for VAP prevention. For instance, probiotics stimulate immune responses by increasing phagocytic cell function and inducing the release of cytokines (interleukin [IL] 1, IL-2, IL-6, IL-12, IL-18, tumor necrosis factor alpha, interferon gamma) and regulate the local inflammatory response by upregulating transforming growth factor beta and IL-10.[34]

In addition, *Lactobacillus* promotes mucin secretion, forms a mucus barrier, and blocks the invasion and adhesion of pathogenic bacteria. Moreover, probiotics synthesize short-chain fatty acids, reduce the intestinal pH, and inhibit the colonization of acid-resistant pathogens, for example, *Pseudomonas aeruginosa*.[10] Furthermore, high safety, low cost, and low toxicity are also advantages of probiotics. For VAP prevention, the specific probiotic strains, patient population, optimal dosage, and proper administration are still challenges that need to be considered in further research; however, our meta-analysis may provide some meaningful clues. Probiotics seem to reduce VAP incidence caused by *P. aeruginosa,* although this finding is tempered by the fact that *P. aeruginosa* is the most common Gram-negative pathogen associated with VAP. Moreover, Synbiotic 2000Forte may be an effective probiotic strain to prevent VAP.

There may be a certain amount of heterogeneity in our study. The strong influences were the differences in the trial

Table 4. Subgroup Analysis for the Primary Outcome

Outcome or Subgroup Title	Studies, no.	Subjects, *N*	Effect Size
Different species of probiotics	13	1,875	0.62 (0.45, 0.85)
Lactobacillus rhamnosus GG + Lactobacillus casei + Lactobacillus acidophilus + Bifidobacterium bifidum	1	149	1.56 (0.74, 3.30)
Lactobacillus casei rhamnosus	2	340	0.62 (0.21, 1.81)
Synbiotic 2000Forte	4	509	0.52 (0.30, 0.90)
Lactobacillus plantarum 299	2	385	0.84 (0.42, 1.68)
L. casei	1	150	0.76 (0.37, 1.57)
Bifidobacterium breve + L. casei	1	72	0.18 (0.06, 0.55)
Bifidobacterium longum + Lactobacillus bulgaricus + Streptococcus thermophilus	1	35	0.36 (0.09, 1.43)
Live *Bacillus subtilis + Enterococcus faecalis*	1	235	0.56 (0.33, 0.95)
Different diagnostic criteria for VAP	13	1,875	0.62 (0.45, 0.85)
Microbiologically confirmed diagnosis	7	986	0.82 (0.55, 1.22)
Clinical diagnosis	4	704	0.57 (0.40, 0.80)
Inexplicit diagnostic criteria	2	185	0.22 (0.10, 0.50)
Different trial design	13	1,875	0.62 (0.45, 0.85)
Double-blind studies	6	885	0.72 (0.44, 1.19)
Single-blind studies	3	220	0.25 (0.13, 0.51)
Open-label studies	4	770	0.68 (0.47, 0.97)
Different pathogens	8	5,605	0.71 (0.54, 0.92)
Acinetobacter baumannii	7	989	0.73 (0.42, 1.25)
Enterobacteriaceae	7	1,119	0.97 (0.54, 1.76)
Haemophilus influenzae	2	397	0.33 (0.05, 2.14)
Klebsiella pneumoniae	4	345	0.81 (0.24, 2.66)
Methicillin-resistant Staphylococcus aureus	4	782	0.69 (0.31, 1.53)
Pseudomonas aeruginosa	8	1,191	0.54 (0.32, 0.91)
Stenotrophomonas maltophilia	4	782	0.70 (0.17, 2.97)

Odds ratio, Mantel-Haenszel, random, with 95% CI used as the statistical method.

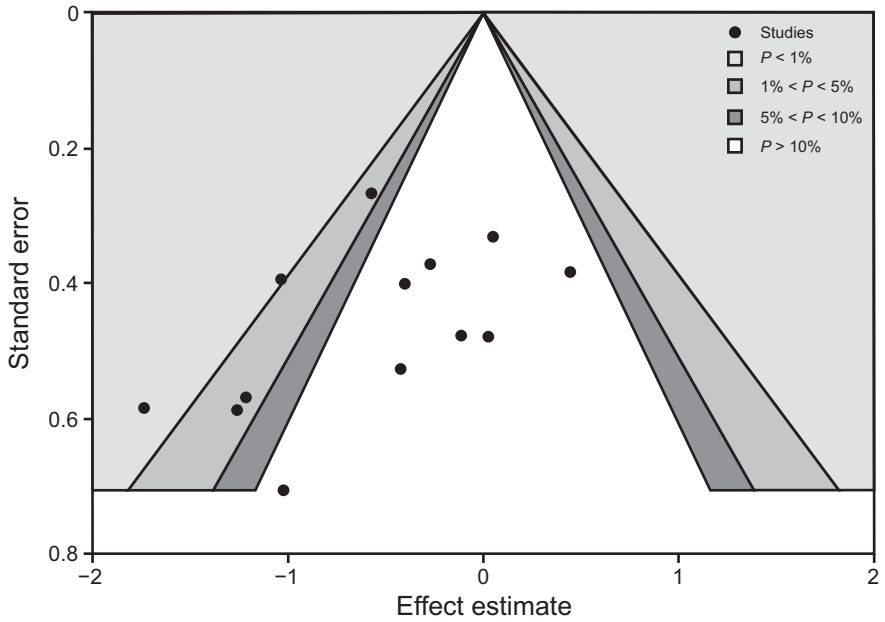

Fig. 12. A contour-enhanced meta-analysis funnel plot for ventilator-associated pneumonia incidence.

design and VAP diagnostic criteria. We performed subgroup analyses based on the differences in trial design and diagnostic criteria. However, the subgroup analysis of the double-blind studies showed no effect of probiotics compared with controls on the prevention of VAP, which meant that the role of probiotics may be inflated by studies with flawed designs. Likewise, no significant difference was found in the subgroup in which VAP was confirmed microbiologically. Different diagnostic criteria may affect the authenticity of the clinical results; hence, explicit and consistent diagnostic criteria are necessary in future studies. In addition, the analysis involved subjects with a variety of diseases, such as trauma, surgery, and sepsis, which may have led to different risks of VAP infection. The difference between probiotic strains, dosages, and delivery methods also reduced the comparability among the studies.

There were other limitations to our study. Due to the restriction of the retrieval strategy, we could not retrieve all documents that met the inclusion criteria. In addition, the methodological quality of the included studies was low, with several RCTs clearly lacking illustrations of randomness, allocation concealment, and blinding, among other factors, which increased difficulty in the risk of bias assessment. Moreover, our conversion of missing means and SDs may have led to inaccurate extrapolation values. Also, significant potential publication bias existed in the study.

Conclusions

An analysis of the available evidence demonstrated that probiotics can reduce the incidence of VAP. Further large-scale, well-designed clinical trials are required to validate this effect and to determine the optimum probiotic strains for the prevention of VAP.

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