



Clinical Benefits From Administering Probiotics to Mechanical Ventilated Patients in Intensive Care Unit: A PRISMA-Guided Meta-Analysis

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Background: The use of probiotics has been considered as a new intervention for ventilator-associated pneumonia (VAP) prevention in the intensive care unit (ICU). The aim of this meta-analysis was to evaluate the effect of probiotics on mechanical-ventilated patients in ICU.

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Song H, Hu W, Zhou X, Tao J, Zhang S, Su X and Wu W (2022) Clinical Benefits From Administering Probiotics to Mechanical Ventilated Patients in Intensive Care Unit: A PRISMA-Guided Meta-Analysis. Front. Nutr. 8:798827. doi: 10.3389/fnut.2021.798827 **Methods:** PubMed, Embase, Scopus, and the Cochrane Library were searched for relevant randomized controlled trials (RCTs) from their respective inception through October 10, 2021. All studies meeting the inclusion criteria were selected to evaluate the effect of probiotics on patients receiving mechanical ventilation in ICU.

Results: A total of 15 studies involving 4,693 participants met our inclusion criterion and were included in this meta-analysis. The incidence of VAP in the probiotic group was significantly lower (odds ratio [*OR*] 0.58, 95% *Cl* 0.41 to 0.81; p = 0.002; $l^2 = 71\%$). However, a publication bias may be present as the test of asymmetry was significant (p = 0.007). The probiotic administration was associated with a significant reduction in the duration of mechanical ventilation (mean difference [*MD*] -1.57, 95% *Cl* -3.12 to -0.03; p = 0.05; inconsistency [$l^2 = 80\%$), length of ICU stay (MD -1.87, 95% *Cl* -3.45 to -0.28; p = 0.02; $l^2 = 76\%$), and incidence of bacterial colonization (*OR* 0.59, 95% *Cl* 0.45 to 0.78; p = 0.0001; $l^2 = 34\%$). Moreover, no statistically significant differences were observed regarding the incidence of diarrhea (*OR* 0.90, 95% *Cl* 0.65 to 1.25; p = 0.54; $l^2 = 12\%$) and mortality (*OR* 0.91, 95% *Cl* 0.80 to 1.05; p = 0.19; $l^2 = 0\%$) between probiotics group and control group.

Conclusion: Our meta-analysis shows that probiotics are associated with a reduction in VAP, as well as the duration of mechanical ventilation, ICU length of stay, and bacterial colonization, but no significant effects on ICU mortality and occurrence of diarrhea. However, in consideration of the significant heterogeneity and publication bias, our findings need to be further validated.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, identifier: CRD42020150770.

Keywords: ventilator-associated pneumonia, probiotics, meta-analysis, intensive care unit, mechanical ventilation, critical care, randomized control trial (RCT)

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is defined as an infectious inflammatory reaction of the lung parenchyma that occurs after mechanical ventilation for more than 48 h (1), which is a common and severe complication in the intensive care unit (ICU). It is reported that VAP affects between 5 and 40% of patients receiving invasive mechanical ventilation, with large variations depending upon the country, ICU type, and criteria used to identify VAP (2). Despite recent advances in the diagnosis and treatment of VAP, it remains one of the most serious problems in the ICU, with a prolonged duration of mechanical ventilation, increased length of ICU and hospital stays, increased cost, and higher mortality risk (3-5). Although many VAP prevention strategies applications are currently available, some strategies have been challenged and the results of clinical trials are disappointing (2, 6). Consequently, despite the epidemiology and diagnostic criteria for VAP are still controversial, and the interpretation of treatment and prevention is still complicated, it is imperative to find new prevention strategies.

Probiotics are a class of active microorganisms that can produce positive effects in the host when administered at the appropriate dosage (7, 8). They can selectively modulate the growth of the microbiome, inhibit colonization with invasive pathogens, and improve the microecological balance of the host (9). Recent meta-analyses (10–13) indicated that the administration of probiotics could significantly reduce the incidence of VAP. However, several studies have described the potential risks of probiotics such as systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, gene transfer, and gastrointestinal side effects (14, 15). Due to the lack of large-scale and high-quality randomized controlled trials (RCTs), whether probiotics have beneficial effects on VAP prevention remains controversial.

Recently, Johnstone et al. completed the largest randomized trial to compare the effect of probiotics on preventing VAP in critically ill patients (16). In this multicenter and pragmatic study concerning 2,650 participates, as compared with placebo, the administration of 1×10^{10} colony forming units of *Lactobacillus rhamnosus* GG two times daily for 9 days did not significantly reduce the risk of VAP. Therefore, this meta-analysis aimed to elucidate the latest and most convincing evidence about the effects of probiotics on VAP prevention in ICU patients receiving mechanical ventilation.

METHODS

Data Sources and Study Selection

This meta-analysis was performed according to the PRISMA statement (17) (checklist in **Supplementary Material 1**). The study protocol has been registered in PROSPERO (CRD 42020150770). PubMed, Embase, Scopus, and Cochrane Library were searched for eligible studies up to October 10, 2021. Search terms included "ventilator-associated pneumonia," "VAP," "probiotics," and "critically ill" relevant studies were enrolled in the present study. The search was limited to studies published

in English. The detailed search strategies were recorded in **Supplementary Material 2**.

Eligibility Criteria

Study inclusion criteria were as follows: (1) Population: Critically ill patients receiving mechanical ventilation; (2) Intervention: Probiotics; (3) Comparison: Placebo or no drug infusion; (4) Outcomes: primary outcomes were incidence of VAP and overall mortality (ICU, hospital, 28-day mortality). Secondary outcomes were ICU and hospital length of stay, duration of mechanical ventilation, incidence of diarrhea, and incidence of bacterial colonization; (5) Design: randomized controlled trial.

Data Extraction and Quality Assessment

Two authors (HS and WH) independently retrieved and extracted relevant studies. The basic characteristics of included studies (first author, years of publication, study design, population, intervention and control methods, and definition of VAP) were recorded in **Table 1**. Any discrepancies in all phases were ultimately resolved through team consensus. Two authors (WH and XZ) independently assessed the risk of bias according to the Cochrane risk of bias tool (32). The evaluation criteria were based on sample selection, allocation concealment (selection bias), blinding of participants and personnel (performance bias), incomplete outcome data (attrition bias), statistical analysis, and outcome validation, selective reporting, and free of the source of funding (reporting bias) measured the degree of bias, the definition of inclusion and exclusion criteria. They were categorized as low risk, high risk, and unclear risk.

Statistical Synthesis and Analysis

Dichotomous data were presented as odds ratio (*OR*) with 95% *CI*; continuous data were presented as mean difference (*MD*) with 95% *CI* for heterogeneity between studies were tested by the Chi-squared test with significance set at a *p*-value of 0.1, and quantitatively by inconsistency (I^2) statistics (33). Significant heterogeneity was suggested when I^2 value >50% and a random-effect model was used. In addition, the funnel plot and Egger's regression test were employed to investigate potential publication bias.

For the incidence of VAP, a predefined subgroup analysis was performed by the definition of VAP (quantitative microbiological confirmed VAP vs. non-quantitative microbiological confirmed VAP). The number needed to treat (NNT) analysis (34) was performed for the incidence of VAP, mortality, diarrhea, and bacterial colonization to provide an understanding of the incremental benefits of administering probiotics relative to usual care. Furthermore, a sensitivity analysis was employed to examine the effect of individual studies by omitting each one at a time.

RESULTS

Study Characteristics

A total of 199 studies were initially retrieved from the above databases, such as 44 from Pubmed, 60 from Embase, 57 from Scopus, and 38 from Cochrane library. Ninety-five were

 TABLE 1 | Characteristics, designs, intervention, and control of the included studies.

Study and year	Design and country	N	Population	Intervention	Control	Definition of ventilator-associated pneumonia
Johnstone et al. (16)	Double blinded, multicenter, in Canada, the United States, and Saudi Arabia	2,653	Critically ill adult patients	1 × 10 ¹⁰ colony forming units of <i>Lacticaseibacillus rhamnosus</i> twice daily for a median of 9-day period	Placebo	New, progressive, or persistent radiographic infiltrate on chest radiograph after at least 2 days of mechanical ventilation, plus any 2 of the following: fever or hypothermia, leukocytosis or leukopenia, and purulent sputum
Mahmoodpoor et al. (18)	Double blinded, multicenter, in Iran	102	Critically ill adult patients	2 capsules of probiotic preparation via feeding tube daily for 14 days. Each capsule contained 10 ¹⁰ bacteria consisting of <i>Lactobacillus</i> , <i>Bifdobacterium</i> and <i>Streptococcus</i> <i>thermophiles</i>	Placebo	Quantitative bronchoalveolar lavage fluid culture
Klarin et al. (19)	No blinded, multicenter, in Sweden	137	Critically ill adult patients	10 ml of a solution containing a total of 10 ¹⁰ CFU of <i>Lactiplantibacillus paraplantarum</i> in oral care procedure BID, until extubation or ICU discharge	No placebo	Chest radiograph combined with at least three of the other four criteria; a purulent tracheal aspirate; positive culture of tracheal aspirates, fever, leukocytosis or leukopenia
Shimizu et al. (20)	Single blinded, single-center, in Japan	72	Sepsis patients	Yakult BL Seichoyaku (contained 1×10^8 of <i>Bifidobacterium</i> and <i>Lactobacillus</i> per gram) 3g/day via nasal tube, started within 3 days after admission, continued until oral intake	·	Pneumonia arises after endotracheal intubation
Zeng et al. (21)	No blinded, multicenter, in China	235	Critically ill adult patients	0.5g probiotics capsules (each probiotics capsule contained active <i>Bacillus subtilis</i> and <i>Enterococcus faecalis</i> of 4.5 \times 10 ⁹ /0.25 g and 0.5 \times 10 ⁹ /0.25 g) TID, started within 24 h of admission, continued until extubation with a maximum of 14 days	No placebo	Chest radiographs combined with at least two of the following criteria: fever, leukocytosis or leukopenia, purulent tracheal aspirates
Banupriya et al. (22)	No blinded, single-center, in India	142	PICU patients	A capsule 2 billion CFU of <i>Lactobacillus</i> , 1 billion CFU of <i>Bifidobacterium</i> , and 300 million CFU of <i>Streptococcus</i> twice a day via nasogastric tube, started at PICU admission, continued for 7 days	No placebo	Pneumonia developing more than 48 h after endotracheal intubation and initiation of MV
Rongrungruang et al. (23)	No blinded, single-center, in Thailand	150	Critically ill adult patients	8×10^9 CFU of <i>Lactobacillus casei</i> for oral care and 8×10^9 CFU of <i>Lactobacillus casei</i> via enteral feeding once a day for 28 days	No placebo	Chest radiograph in combination with at least 3 of the following 4 criteria: fever, leukocytosis or leukopenia, purulent tracheal aspirate, a semi quantitative culture of tracheal aspirate samples that was positive for pathogenic bacteria
Oudhuis et al. (24)	No blinded, multicenter, in Netherlands	254	Critically ill adult patients	Lactiplantibacillus paraplantarum in a dose of 5×10^9 CFU BID through a nasogastric tube, until ICU discharge, death or extubation		Quantitative culture result in bronchoalveolar lavage fluid
Tan et al. (25)	Single blinded, single-center, in China	52	Adult patients with severe TBI	A total of 109 CFU bacteria a day. Containing 0.5×10^8 <i>Bifidobacterium</i> <i>longum</i> , 0.5×10^7 <i>Lactobacillus</i> <i>bulgaricus</i> , and 0.5×10^7 <i>Streptococcus</i> <i>thermophilus</i> TID via nasogastric tube, started within 48 h after admission, continued for 21 days	No placebo	New or progressive radiographic infiltrate with fever, leukocytosis, leucopenia, or purulent tracheobronchial secretions, and positive semiquantitative cultures of tracheobronchial secretions
Barraud et al. (26)	Double blinded, multicenter, in France	167	Critically ill adult patients	2 × 10 ¹⁰ of revivable bacteria (mainly Lactobacillus rhamnosus, but also Lactobacillus casei, Lactobacillus acidophilus, and Bifidobacterium bifidum) QD via enteral tube for the period of MV (less than 28 days)	Placebo	Chest radiograph, purulent tracheal secretions, fever, leukocytosis, and positive quantitative cultures of distal pulmonary secretions

(Continued)

TABLE 1 | Continued

Study and year	Design and country	Ν	Population	Intervention	Control	Definition of ventilator-associated pneumonia
Morrow et al. (27)	Double blinded, single-center, in the USA	138	Critically ill adult patients	2×10^9 CFU of <i>Lactobacillus rhamnosus</i> BID via nasogastric tube until extubation	Placebo	New and persistent infiltrate on chest radiographs with two of three supporting findings: fever, leukocytosis, and purulent sputum
Knight et al. (28)	Double blinded, single-center, in the UK	259	Critically ill adult patients	1 × 10 ¹⁰ of <i>Pediococcus pentosaceus</i> , <i>Leuconostoc mesenteroides</i> , <i>Lactobacillus paracasei subsp paracasei</i> and <i>Lactiplantibacillus paraplantarum</i> BID via nasogastric or orogastric tube with 24 h of ICU admission, until 28 day after admission	Placebo	Pneumonia occurring more than 48 h after endotracheal intubation
Giamarellos- Bourboulis et al. (29)	Double blinded, multicenter, in Greece	72	Multiple injured patients	Synbiotic preparation consisted of a combination of 10 ¹¹ CFU of each of four probiotics; <i>Pediococcus pentoseceus</i> , <i>Leuconostoc mesenteroides</i> , <i>Lactobacillus paracasei</i> , and <i>Lactiplantibacillus paraplantarum</i> per day for a 15-day study period	Placebo	New or persistent consolidation in lung X-ray, purulent TBS, and clinical pulmonary infection score
Forestier et al. (30)	Double blinded, single-center, in France	208	Critically ill adult patients	10 ⁹ CFU of <i>Lacticaseibacillus rhamnosus</i> BID via nasogastric tube, started at the 3rd day of ICU admission, until ICU discharge or death	Placebo	Positive quantitative sample, abnormal radiographical and progressive parenchymatous infiltrates
Spindler-Vesel et al. (31)	Double blinded, single-center, in Slovenia	55	Multiple injured patients	Synbiotic consisting of 10 ¹⁰ <i>Pediococcus</i> pentosaceus, 10 ¹⁰ <i>Ligilactobacillus</i> araffinosus, 10 ¹⁰ <i>Lactobacillus</i> paracasei subsp paracasei, 10 ¹⁰ <i>Lactiplantibacillus</i> paraplantarum, the study period was 7 days	No placebo	Microbiological specimens

excluded due to duplicates, the remaining 104 articles were screened based upon the review of the titles and abstracts, 67 studies were excluded. Full texts of the remaining 37 articles were independently assessed by two investigators to determine inclusion and exclusion. After strict screening according to inclusion and exclusion criteria, 22 studies were excluded, 15 RCTs (16, 18–31) were finally included in our meta-analysis (**Figure 1** shows the study selection).

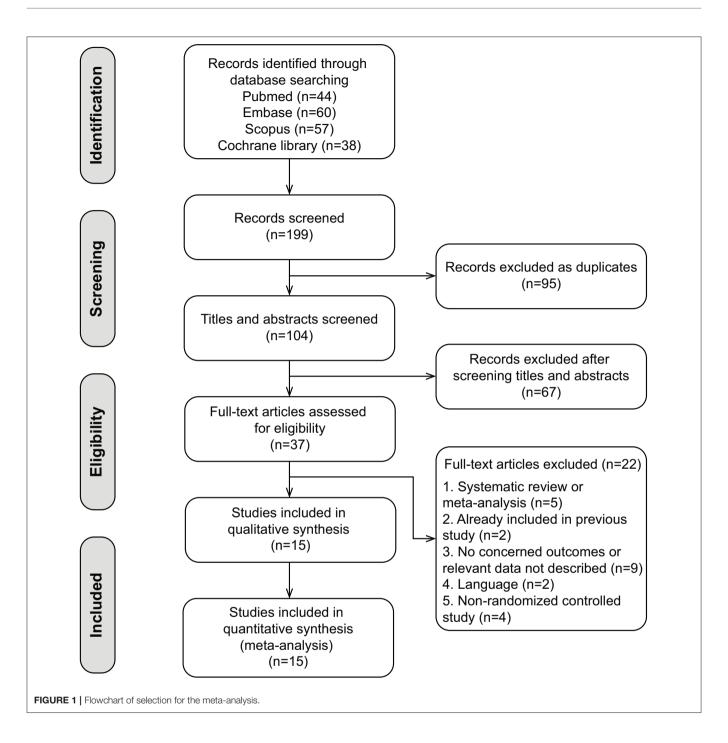
Characteristics of included trials are shown in Table 1. These trials were published between 2007 and 2021. The sample sizes of included trials were ranged from 52 to 2,650 (the total number was 4,693). Most of the studies enrolled a mixed population of critically ill adult patients, three studies (25, 29, 31) included patients with severe trauma, Shimizu et al. (20) focused on patients with sepsis and Banupriya et al. (22) performed their trial in a pediatric intensive care unit. Furthermore, according to the new taxonomic description (35), the species and strain of probiotics administration varied in these studies. In six of the studies (16, 19, 23, 24, 27, 30), a single probiotic was used, such as Lacticaseibacillus rhamnosus (35) in Johnstone et al. (16), Morrow et al. (27), and Forestier et al. (30), Lactiplantibacillus paraplantarum (35) in Klarin et al. (19) and Oudhuis et al. (24), Lactobacillus casei (35) in Rongrungruang et al. (23). The rest of the nine studies used multiple probiotics. Furthermore, only one study (19) administrated probiotics as oral care, whereas others supplemented probiotics through the enteral route. Of the 15

included studies, all the studies reported the incidence of VAP. The incidence of VAP ranged from 7 to 81%, with an average incidence of 24%. In addition, the definition of VAP was varied among all studies, six studies (18, 23–26, 30) used the quantitative microbiological test to define VAP, the rest of included studies used non-quantitative microbiological or clinical features to define VAP.

Quality Assessment

The risk of bias assessment was summarized in **Figure 2**. Six studies did not report the details of random sequence generation and allocation concealment. Seven studies were rated as high risk of bias since five studies (19, 21–24) were open-label trials and two (20, 25) were single-blind trials. Moreover, the blinding method for outcome assessment was not reported in ten studies, which would either underestimate or overestimate the size of the effect. Furthermore, three studies had other biases: Klarin et al. (19) only administrated probiotics as oral care, patients in the control group received selective decontamination of the digestive tract in the trial by Oudhuis et al. (24). Banupriya et al. (22) focused on critically ill children in the pediatric intensive care unit.

The test of asymmetry on the funnel plot and Egger's test was concluded for every outcome (**Supplementary Material 3**). Potential publication bias was observed for the incidence of VAP and diarrhea (Egger's test: p < 0.10), thus, an analysis



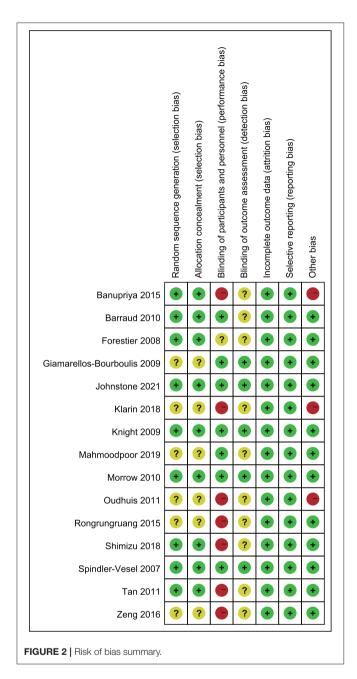
using the trim and fill method was performed. After imputing, the funnel plot became symmetrical and the pooled estimate showed no association between probiotics supplementation and the incidence of VAP (*OR* 0.92, 95% *CI* 0.64 to 1.33) or diarrhea (*OR* 1.08, 95% *CI* 0.80 to 1.46).

Primary Outcome

All included studies with 4,693 participants, 2,338 in the probiotics group, and 2,355 in the placebo group reported the incidence of VAP. The analysis showed that the incidence of VAP

in the probiotic group was significantly lower than that in the control group (*OR* 0.58, 95% *CI* 0.41 to 0.81; p = 0.002; $I^2 = 71\%$; **Figure 3A**). High heterogeneity was seen between studies. The NNT to prevent one patient of VAP in mechanical ventilated patients in ICU was 24.

A total of 14 trials with 4,461 patients were included in the meta-analysis examining the association between mortality and probiotic intervention. The studies were found to be homogenous, there was no significant association between probiotics and mortality (*OR* 0.91, 95% *CI* 0.80 to 1.05; p = 0.19;



 $I^2 = 0\%$; **Figure 3B**). The NNT to prevent one patient of death in mechanical ventilated patients in ICU was 62. Furthermore, there was no significant difference in ICU mortality in the probiotic group vs. the control group (*OR* 0.94, 95% *CI* 0.81 to 1.10; p = 0.47; $I^2 = 0\%$; **Figure 3C**).

Secondary Outcome

Nine studies including 1,977 patients in the probiotics group and 1,995 patients in the control group provided data on the duration of mechanical ventilation. The duration of mechanical ventilation in the probiotics group was shorter than the control group, there was high heterogeneity between the two groups (*MD* -1.57, 95% *CI* -3.12 to -0.03; p = 0.05; $I^2 = 80\%$; **Figure 4A**).

A total of 13 studies with 909 in the probiotics group and 912 in the control group reported the length of ICU stay. Pooled data demonstrated that the length of ICU stay of patients in the probiotics group was shorter than that in the control group, there was high heterogeneity between the two groups (MD - 1.87, 95% CI - 3.45 to -0.28; p = 0.02; $I^2 = 76$ %; **Figure 4B**).

Seven of the 14 studies included 529 patients in probiotics and 529 patients in the control group were pooled to analyze the bacterial colonization, a significant reduction was found in the probiotics group than the control group (*OR* 0.59, 95% <u>*CI*</u> 0.45 to 0.78; p = 0.0001; $I^2 = 34\%$; **Figure 4C**), and a moderate heterogeneity was seen between the studies. The NNT to prevent one patient of bacterial colonization in mechanical ventilated patients in ICU was 9.

Six studies including 888 patients with 444 in the probiotics group and 444 in the control group reported on diarrhea, no statistically significant differences were observed regarding diarrhea between the probiotics group and control group, a low heterogeneity was seen between the studies (*OR* 0.90, 95% *CI* 0.65 to 1.25; p = 0.54; $I^2 = 12\%$; **Figure 4D**). The NNT to prevent one patient of diarrhea in mechanical ventilated patients in ICU was 74.

Subgroup and Sensitivity Analyses

The subgroup analysis of quantitative microbiological confirmed VAP (*OR* 0.72, 95% *CI* 0.39 to 1.30; p = 0.28; $I^2 = 65\%$) showed no significant association between probiotics and VAP. However, the subgroup of non-quantitative microbiological confirmed VAP (*OR* 0.49, 95% *CI* 0.31 to 0.79; p = 0.003; $I^2 = 77\%$) suggested a reduction of VAP incidence. The difference between the subgroups was not statistically significant (p = 0.33; **Figure 5**).

Moreover, according to the average incidence of VAP, patients were divided into the low and high incidence of VAP groups. The effect of probiotics in reducing the incidence of VAP was statistically significant in trials with high incidence of VAP (OR 0.34, 95% CI 0.22 to 0.53; p < 0.001; $I^2 =$ 44%) while it was not significant in those with relatively low incidence of VAP (OR 1.01, 95% CI 0.86 to 1.19; p =0.88; $I^2 = 0\%$; Figure 6). In addition, since only six studies were double-blind trials, subgroup analyses were performed based on the differences in trial design. The subgroup analysis of the double-blind studies showed no effect of probiotics compared with controls on the prevention of VAP (OR 0.62, 95% CI 0.36 to 1.05; p = 0.07; $I^2 = 74\%$; Figure 7), while the subgroup of no-blind studies still showed a protective effect (OR 0.54, 95% CI 0.35 to 0.84; p = 0.006; $I^2 = 57\%$; Figure 7).

Since the clinical features of included population, types and dosage of probiotics, and definition of VAP

	Experim	ental	Contr			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	I M-H, Random, 95% Cl
Banupriya 2015	12	70	35	72	7.1%	0.22 [0.10, 0.47]	
Barraud 2010	23	87	15	80	7.3%	1.56 [0.75, 3.25]	
Forestier 2008	24	102	24	106	8.0%	1.05 [0.55, 2.00]	
Giamarellos-Bourboulis 2009	15	36	16	36	6.1%	0.89 [0.35, 2.27]	
Johnstone 2021	289	1318	284	1332	10.7%	1.04 [0.86, 1.25]	
Klarin 2018	7	69	10	68	5.6%	0.65 [0.23, 1.83]	
Knight 2009	12	130	17	129	7.0%	0.67 [0.31, 1.47]	
Mahmoodpoor 2019	32	48	51	54	4.2%	0.12 [0.03, 0.44]	←
Morrow 2010	17	68	33	70	7.4%	0.37 [0.18, 0.77]	
Oudhuis 2011	10	130	9	124	6.1%	1.06 [0.42, 2.72]	
Rongrungruang 2015	18	75	22	75	7.4%	0.76 [0.37, 1.57]	
Shimizu 2018	5	35	18	37	5.0%	0.18 [0.06, 0.55]	←
Spindler-Vesel 2007	4	26	12	29	4.3%	0.26 [0.07, 0.94]	·
Tan 2011	7	26	13	26	4.9%	0.37 [0.12, 1.17]	
Zeng 2016	43	118	59	117	8.8%	0.56 [0.33, 0.95]	
Total (95% CI)		2338		2355	100.0%	0.58 [0.41, 0.81]	◆
Total events	518		618				
Heterogeneity: Tau ² = 0.28; Ch	i² = 48.52.	df = 14 (P < 0.000)1): ² =	- 71%		

в

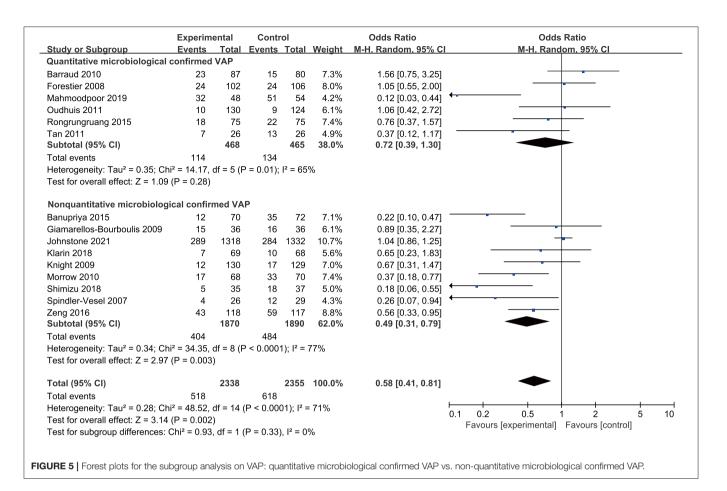
	Experime	ental	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% Cl
Banupriya 2015	17	70	23	72	4.0%	0.68 [0.33, 1.43]	
Barraud 2010	22	87	19	80	3.4%	1.09 [0.54, 2.20]	
Giamarellos-Bourboulis 2009	5	36	10	36	2.0%	0.42 [0.13, 1.38]	
Johnstone 2021	363	1318	381	1332	63.3%	0.95 [0.80, 1.12]	
Klarin 2018	14	69	12	68	2.2%	1.19 [0.50, 2.80]	
Knight 2009	34	130	42	129	7.2%	0.73 [0.43, 1.26]	
Mahmoodpoor 2019	5	48	6	54	1.2%	0.93 [0.26, 3.27]	
Morrow 2010	12	68	15	70	2.8%	0.79 [0.34, 1.83]	
Oudhuis 2011	34	130	32	124	5.6%	1.02 [0.58, 1.78]	
Rongrungruang 2015	18	75	17	75	3.0%	1.08 [0.51, 2.30]	
Shimizu 2018	3	35	4	37	0.8%	0.77 [0.16, 3.73]	
Spindler-Vesel 2007	2	26	2	29	0.4%	1.13 [0.15, 8.61]	
Tan 2011	3	26	5	26	1.0%	0.55 [0.12, 2.58]	
Zeng 2016	11	103	16	108	3.2%	0.69 [0.30, 1.56]	
Total (95% CI)		2221		2240	100.0%	0.91 [0.80, 1.05]	•
Total events	543		584				
Heterogeneity: Chi ² = 5.07, df =	13 (P = 0.9)	97); l² =	0%				
Test for overall effect: Z = 1.33 ((P = 0.19)						0.1 0.2 0.5 1 2 5 1 Favours [experimental] Favours [control]

S Total 1 87 9 1318 0 69 3 130 5 48 4 130	Events 21 296 11 35 6 32	80 1332 68 129 54	Weight 5.1% 71.9% 2.9% 8.5% 1.6%	M-H, Fixed, 95% Cl 0.89 [0.44, 1.80] 0.94 [0.78, 1.13] 0.88 [0.35, 2.23] 0.74 [0.42, 1.30] 0.93 [0.26, 3.27]	M-H. Fixed, 95% Cl
9 1318 0 69 3 130 5 48	296 11 35 6	1332 68 129 54	71.9% 2.9% 8.5%	0.94 [0.78, 1.13] 0.88 [0.35, 2.23] 0.74 [0.42, 1.30]	
0 69 3 130 5 48	11 35 6	68 129 54	2.9% 8.5%	0.88 [0.35, 2.23] 0.74 [0.42, 1.30]	
3 130 5 48	35 6	129 54	8.5%	0.74 [0.42, 1.30]	
5 48	6	54			
	-		1.6%	0.93 [0.26, 3.27]	
4 130	32				
	02	124	7.5%	1.02 [0.58, 1.78]	
5 118	9	117	2.4%	1.75 [0.73, 4.17]	
1900		1904	100.0%	0.94 [0.81, 1.10]	•
2	410				
= 6 (P = 0.	84); l² = 0)%			
(P = 0.47)				0.1 0.2 0.5 1 2 5 10 Favours [experimental] Favours [control]
1	1900 2 = 6 (P = 0.	1900 2 410	1900 1904 2 410 = 6 (P = 0.84); I ² = 0%	$1900 1904 100.0\%$ $2 410$ $= 6 (P = 0.84); I^2 = 0\%$	1900 1904 100.0% 0.94 [0.81, 1.10] 2 410 = 6 (P = 0.84); l ² = 0%

FIGURE 3 | Forest plots for the effect of probiotics on (A) incidence of VAP; (B) overall mortality; (C) ICU mortality.

were not constant among included studies, a sensitivity analysis was performed by removing each trial to examine the effect of individual study on the overall result. The sensitivity analysis showed similar results to the overall analysis, indicating good robustness (**Supplementary Material 4**).

ExperimentalControlOdds RatioOdds RatioStudy or SubgroupEventsTotalEventsTotalWeightM-H. Fixed. 95% CIBanupriya 20152470377217.1% $0.49 [0.25, 0.97]$ M-H. Fixed. 95% CIBarraud 20104874802.8% $0.92 [0.22, 3.79]$ M-H. Fixed. 95% CIKlarin 20183169296811.5% $1.10 [0.56, 2.16]$ M-H. Fixed. 95% CIMahmoodpoor 20192348345411.9% $0.54 [0.25, 1.19]$ Morrow 20102668497021.3% $0.27 [0.13, 0.54]$ Zeng 2016551186611725.3% $0.67 [0.40, 1.13]$ Total (95% CI)529529100.0%0.59 [0.45, 0.78]Total events215275Heterogeneity: Chi² = 9.14, df = 6 (P = 0.17); l² = 34%Test for overall effect: Z = 3.82 (P = 0.0001)	a. 1 a :		eriment			ontrol			Mean Difference	Mean Difference
$ \begin{array}{c} \text{derivative} 2021 & \text{B} & 0.68 & 138 & 0.68 & 1392 & 188\% & 0.00 [1.65] (0.51] \\ \text{Kingh 2026 } & \text{S.5} & 5.25 & 130 & 6.33 & 6 & 129 & 151\% & -1.00 [1.28, 0.67] (0.51] \\ \text{Kingh 2026 } & \text{S.7} & $								-		
Kim 2018 9.0 16.22 60 8.09 16.00 68 0.0% 0.0 14.85.67 Malmodopor 2018 8.75 4.79 44 12.08 7.43 54 12.3% 3.31 6.87.09 Malmodopor 2018 8.75 4.79 44 12.08 7.43 54 12.3% 3.31 6.87.09 Malmodopor 2018 1.5 6.49 4.0 12.08 7.13 54 12.3% 3.31 6.87.09 Malmodopor 2018 1.5 6.49 4.0 12.08 7.13 54 12.3% 3.31 6.87.09 Syndher Vseit 2007 12.1 8.86 28 11.57 5.46 129 0.11 (3.8, 5.61, 4.37) Zeng 2016 13 9.76 118 19.33 11.26 117 11.3% 6.33 (4.03, 3.63) Total (95.5 C) 13 9.76 118 19.33 11.26 117 11.3% 6.33 (4.03, 3.63) Total (95.5 C) 107 - 0.32, d7 = 0.00011; P = 80% Test to ownell effect 2.2 2.00 (P = 0.03) B Suck or Subgroup Man. 50 Total Man. 50 Total Mal. 50 T										
$\begin{aligned} & \text{Kulpt 2006} & \text{S.3} & \text{S.2} & \text{130} & \text{S.3} & \text{S.2} & \text{130} & \text{S.3} & \text{S.7} &$										
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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Morrow 2010	9.5	6.3	68	9.6	7.2	70	12.6%	-0.10 [-2.36, 2.16]	
$\frac{2 \text{ org} 2016}{\text{Total} (95\% (c))} = 3.61; Ch^{-2} = 0.32; df = 6[2 + 0.00001]; l^{-2} = 05\% (c) = 0.0001; l^{-2} = 05\% (c) = 0.0001]; l^{-2} = 05\% (c) = 0.0001]; l^{-2} = 05\% (c) = 0.00001; l^{-2} = 0.000001; l^{-2} = 0.00001; l^{$	Oudhuis 2011	16.7	23.6	130	14.1	17.2	124	6.0%	2.60 [-2.46, 7.66]	
Total (95% CI) Heterogeneity: Tau" = 3.81; Chi" = 40.32, df = 8 (P = 0.00001); P = 80% Test for overall effect: Z = 0.0 (P = 0.05) 3 5 5 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 11 10 11 10 11 10 11 10 11 10 11 10 11 10 11 10 11 11 11 10 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11 11	Spindler-Vesel 2007	12.1	8.86	26	11.67	5.46	29	8.1%	0.43 [-3.51, 4.37]	
Heterogeneration, Taur = 2.81; Chrl = 40.32, df = 8 (P < 0.0001); P = 80% Test for overall effect; Z = 2.00 (P = 0.05) 3 3 3 3 3 3 3 3	Zeng 2016	13	9.76	118	19.33	11.26	117	11.3%	-6.33 [-9.03, -3.63]	
Test for overall effect: Z = 2.00 (P = 0.05) 10 Sudy or Subgroup Keperimental Control Mean Difference Mean Difference Sudy or Subgroup Man SD Total Mean SD Total Mean Mean Difference Barrau 2010 18 Sudy or Subgroup Mean Difference Mean Difference No find Weigh Mean Difference Mean Difference	Total (95% CI)			1977			1995	100.0%	-1.57 [-3.12, -0.03]	•
Favours [experimental Favours [experimental] Favours [experimental] Favours [experimental] Favours [experimental] B Study or Subgroup Mean SD Total Weight Mean Difference Burger Subgroup Mean SD Total Weight Mean Difference Subgroup Mean Difference Mean Difference Subgroup Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Difference Mean Dif	Heterogeneity: Tau ² =	3.61; Ch	ni² = 40.3	32, df =	= 8 (P <	0.00001); ² =	80%		
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Tan 2011 6.8 3.8 26 10.7 7.3 26 8.7% -3.80 [7.06, -0.74] Zeng 2016 21.33 13.51 118 29.67 33.78 117 4.0% -8.34 [14.93, -1.75] Total (95% CI) 2227 2244 100.0% -1.87 [-3.45, -0.28] Heterogeneity: Tau ² = 4.92; Ch ² = 50.37, df = 12 (P < 0.00001); P = 76% Test for overall effect: Z = 2.31 (P = 0.02) C C Experimental Control Odds Ratio Study or Subgroup Events Total Events Total Weight M-H. Fixed, 95%, Cl Barraud 2010 4 87 4 80 2.8% 0.92 [0.22, 3.79] Klain 2018 31 69 29 68 11.5% 1.10 [0.56 2.16] Knight 2009 52 69 56 68 9.9% 0.66 [0.29, 1.50] Mahmoodpoor 2019 23 48 34 54 11.9% 0.54 [0.25, 1.19] Morrow 2010 28 68 49 70 2.1.3% 0.67 [0.40, 1.13] Total (95% CI) 529 529 100.0% 0.59 [0.45, 0.76] Total events 215 275 Heterogeneity: Ch ² = 9.14, df = 6 (P = 0.17); P = 34% Test for overall effect: Z = 3.82 (P = 0.0001) Experimental Control Odds Ratio Odds Ratio Study or Subgroup Events Total Events Total Weight M-H. Fixed, 95%, Cl Heterogeneity: Ch ² = 9.14, df = 6 (P = 0.17); P = 34% Test for overall effect: Z = 3.82 (P = 0.0001) C Study or Subgroup Events Total Events Total Weight M-H. Fixed, 95%, Cl Barraud 2010 48 87 42 80 25.9% 1.11 [0.61, 2.05] Giamarellos-Bourboulis 2009 5 36 10 36 11.4% 0.42 [0.13, 1.38] Norrow 2010 42 88 44 70 2.1.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 147 19 12% 11.3% 0.76 [0.27, 2.10] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16, 1.20] Mahmoodpoor 2019 7 148 15 54 15.9% 0.44 [0.16										
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Heterogeneity: Tau ² = 4.92; Chi ² = 50.37, df = 12 (P < 0.00001); P = 76% Test for overall effect: Z = 2.31 (P = 0.02) C C Study or Subgroup Events Total Events Total Weight M-H. Fixed. 95% Cl Banupriya 2015 24 70 37 72 17.1% 0.49 (0.25, 0.97) Barraud 2010 4 87 4 80 2.8% 0.92 (0.22, 3.79) Klarin 2018 31 69 29 68 11.5% 1.10 (0.56, 2.16) Mahmoodpoor 2019 23 48 34 54 11.9% 0.65 (0.22, 1.50) Mahmoodpoor 2019 23 48 34 54 11.9% 0.65 (0.22, 1.19) Morrow 2010 26 68 49 970 21.3% 0.27 (0.13, 0.54) Zeng 2016 555 118 66 117 25.3% 0.67 [0.40, 1.13] Total (95% Cl) 529 529 100.0% 0.59 [0.45, 0.78] Total events 215 275 Heterogeneity: Chi ² = 9.14, df = 6 (P = 0.17); P = 34% Test for overall effect: Z = 3.82 (P = 0.0001) C Study or Subgroup Events Total Events Total Weight M-H. Fixed. 95% Cl Barraud 2010 48 87 42 80 2259 110.0% Odds Ratio Odds Ratio Odds Ratio Odds Ratio Odds Ratio Odds Ratio 0.1 0.2 0.5 1 2 5 10 Favours [experimental] Favours [experimental] Favours [experimental] Favours [experimental] Favours [experimental] Favours [experimental] Favours [experimental] Total events 1215 275 Heterogeneity: Chi ² = 9.14, df = 6 (P = 0.17); P = 34% Test for overall effect: Z = 3.82 (P = 0.0001) C Study or Subgroup Events Total Events Total Weight M-H. Fixed. 95% Cl M-H. Fixed. 95	Total (95% CI)			2227			2244	100.0%	-1.87 [-3.45, -0.28]	•
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Heterogeneity: $Chi^2 = 5.66$, $df = 5$ (P = 0.34); $l^2 = 12\%$ Image: the second	Study or Subgroup Banupriya 2015 Barraud 2010 Klarin 2018 Knight 2009 Mahmodpoor 2019 Morrow 2010 Zeng 2016 Total (95% CI) Total events Heterogeneity: Chi ² Test for overall effect Study or Subgroup Barraud 2010 Giamarellos-Bourbou Knight 2009 Mahmodpoor 2019 Morrow 2010 Rongrungruang 2015	Exp	erimen nts - 24 4 31 52 23 26 55 215 215 215 215 215 215 215	Ital 70 87 69 48 68 118 529 2 = 0.1 6000000000000000000000000000000000000	Events 37 29 56 34 46 66 275 17); ² = 11) tal Fotal E 87 36 130 48 68 75	Tota 7 72 800 66 61 54 5 117 529 700 334% 200 0 9 10 9 15 44	2 177 2 27 3 111 3 9 4 11 0 21 7 25 3 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 10	.1% .8% .5% .9% .3% .3% .0% .0% .0% .10% 11.4% 11.3% 15.9% 21.9% 13.8%	-H, Fixed. 95% Cl 0.49 [0.25, 0.97] 0.92 [0.22, 3.79] 1.10 [0.56, 2.16] 0.66 [0.29, 1.50] 0.54 [0.25, 1.19] 0.27 [0.13, 0.54] 0.67 [0.40, 1.13] 0.59 [0.45, 0.78] M-H, Fixed. 95% Cl 1.11 [0.61, 2.05] 0.42 [0.13, 1.38] 0.76 [0.27, 2.10] 0.44 [0.16, 1.20] 0.95 [0.48, 1.90] 1.48 [0.68, 3.22]	Favours [experimental] Favours [control]
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Favours [experimental] Favours [control]	Study or Subgroup Banupriya 2015 Barraud 2010 Klarin 2018 Knight 2009 Mahmoodpoor 2019 Morrow 2010 Zeng 2016 Total (95% CI) Total events Heterogeneity: Chi ² Test for overall effect D Study or Subgroup Barraud 2010 Giamarellos-Bourbou Knight 2009 Mahmoodpoor 2019 Morrow 2010 Rongrungruang 2015 Total (95% CI) Total events	Exp	erimen 24 4 31 52 23 26 55 215 cf = 6 (F 82 (P = Expe Even	Ital 70 87 69 48 68 118 529 $2 = 0.20$ 0.0000 0.0000 0.0000 69 48 5 7 42 19 28	Events 37 29 56 34 49 66 275 17); ² = 11) tal Fotal E 87 36 130 48 68 75 444	Total 7 72 800 66 62 62 5 54 5 54 6 117 529 34% Control 9 15 44 14 134 134	2 177 2 27 3 111 3 9 4 11 0 21 7 25 3 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 10	.1% .8% .5% .9% .3% .3% .0% .0% .0% .10% 11.4% 11.3% 15.9% 21.9% 13.8%	-H, Fixed. 95% Cl 0.49 [0.25, 0.97] 0.92 [0.22, 3.79] 1.10 [0.56, 2.16] 0.66 [0.29, 1.50] 0.54 [0.25, 1.19] 0.27 [0.13, 0.54] 0.67 [0.40, 1.13] 0.59 [0.45, 0.78] M-H, Fixed. 95% Cl 1.11 [0.61, 2.05] 0.42 [0.13, 1.38] 0.76 [0.27, 2.10] 0.44 [0.16, 1.20] 0.95 [0.48, 1.90] 1.48 [0.68, 3.22]	Favours [experimental] Favours [control]
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	Study or Subgroup Banupriya 2015 Barraud 2010 Klarin 2018 Knight 2009 Mahmoodpoor 2019 Morrow 2010 Zeng 2016 Total (95% CI) Total events Heterogeneity: Chi ² Test for overall effect D <u>Study or Subgroup</u> Barraud 2010 Giamarellos-Bourbou Knight 2009 Mahmoodpoor 2019 Morrow 2010 Rongrungruang 2015 Total (95% CI) Total events Heterogeneity: Chi ² =	Exp	erimen ints 24 4 31 52 23 26 55 215 cff = 6 (F 82 (P = Expe Even	Ital 70 87 69 48 68 118 529 2 = 0.1 6000000000000000000000000000000000000	Events 37 29 56 34 49 66 275 17); ² = 11) tal Fotal E 87 36 130 48 68 75 444	Total 7 72 800 66 62 62 5 54 5 54 6 117 529 34% Control 9 15 44 14 134 134	2 177 2 27 3 111 3 9 4 11 0 21 7 25 3 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 50 100 10	.1% .8% .5% .9% .3% .3% .0% .0% .0% .10% 11.4% 11.3% 15.9% 21.9% 13.8%	-H, Fixed. 95% Cl 0.49 [0.25, 0.97] 0.92 [0.22, 3.79] 1.10 [0.56, 2.16] 0.66 [0.29, 1.50] 0.54 [0.25, 1.19] 0.27 [0.13, 0.54] 0.67 [0.40, 1.13] 0.59 [0.45, 0.78] M-H, Fixed. 95% Cl 1.11 [0.61, 2.05] 0.42 [0.13, 1.38] 0.76 [0.27, 2.10] 0.44 [0.16, 1.20] 0.95 [0.48, 1.90] 1.48 [0.68, 3.22]	Favours [experimental] Favours [control]



DISCUSSION

Our meta-analysis included a total of 15 trials with 4,693 participants to evaluate the effect of probiotics in the prevention of VAP. As far as we know, this is the largest and most updated meta-analysis to evaluate the effects of probiotics in critically ill patients. The analysis of the outcomes demonstrated that probiotics significantly decreased the incidence of VAP, and the NNT to prevent one patient of VAP in mechanical ventilated patients in ICU was 24. Moreover, the use of probiotics in mechanical ventilated patients was associated with reduced duration of mechanical ventilation, length of ICU stay, and bacteria colonization. However, no appreciable effects were conferred by probiotics on ICU mortality and the occurrence of diarrhea. Moreover, subgroup analysis showed that the definition of VAP was a potential source of heterogeneity. The probiotic had no significant effect on the quantitative microbiological confirmed VAP, while the protective effect was proved by other RCTs which used the non-quantitative microbiological method to define VAP. Thus, to further evaluate the effect of probiotics in VAP, large scale multicentric studies with a unified definition of VAP are needed in the future. In addition, in patients with a high risk of developing VAP, the beneficial effect of probiotics in reducing the incidence of VAP was more significant, while there was no significant difference in patients with a low incidence of VAP.

Our results are generally consistent with the recent metaanalyses (10-13) that the use of probiotics was associated with a significant reduction in VAP but no significant difference in overall mortality. However, our results demonstrated that the use of probiotics could reduce the length of ICU stay, duration of mechanical ventilation, and incidence of bacteria colonization. Compared with previous meta-analyses by Zhao et al. (13) and Ji et al. (12), the trials by Klarin et al. (36) and Kotzampassi et al. (37) were excluded in our meta-analysis because the patient cohorts of these two trials are the same as in Klarin et al. (19) and Giamarellos-Bourboulis et al. (29). Furthermore, our meta-analysis included the latest study by Johnstone et al. (16), which enrolled more than 2,600 patients and was the largest randomized trial to date. In addition, our meta-analysis also found the beneficial effect of probiotics in reducing the incidence of VAP was more effective in patients at high risk of developing into VAP. It is a common complication in patients receiving invasive mechanical ventilation, identifying effective preventive measures of VAP is difficult because of the many factors involved and the lack of a unique definition. Even though two meta-analyses did not find a statistically significant

Study or Subgroup	Experim						
Study or Subaroup			Contr			Odds Ratio	Odds Ratio
Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% Cl
Low							
Barraud 2010	23	87	15	80	7.3%	1.56 [0.75, 3.25]	
Forestier 2008	24	102	24	106	8.0%	1.05 [0.55, 2.00]	
Johnstone 2021	289	1318	284	1332	10.7%	1.04 [0.86, 1.25]	+
Klarin 2018	7	69	10	68	5.6%	0.65 [0.23, 1.83]	
Knight 2009	12	130	17	129	7.0%	0.67 [0.31, 1.47]	
Oudhuis 2011	10	130	9	124	6.1%	1.06 [0.42, 2.72]	· · · · ·
Rongrungruang 2015	18	75	22	75	7.4%	0.76 [0.37, 1.57]	
Subtotal (95% CI)		1911		1914	52.1%	1.01 [0.86, 1.19]	•
Total events	383		381				
Heterogeneity: Tau ² = 0.00; Ch	ni² = 3.75, d	f = 6 (P	= 0.71); l ²	² = 0%			
Test for overall effect: Z = 0.15	(P = 0.88)						
High							
Banupriya 2015	12	70	35	72	7.1%	0.22 [0.10, 0.47]	
Giamarellos-Bourboulis 2009	15	36	16	36	6.1%	0.89 [0.35, 2.27]	
Mahmoodpoor 2019	32	48	51	54	4.2%	0.12 [0.03, 0.44]	
Morrow 2010	17	68	33	70	7.4%	0.37 [0.18, 0.77]	
Shimizu 2018	5	35	18	37	5.0%	0.18 [0.06, 0.55]	
Spindler-Vesel 2007	4	26	12	29	4.3%	0.26 [0.07, 0.94]	
Tan 2011	7	26	13	26	4.9%	0.37 [0.12, 1.17]	
Zeng 2016	43	118	59	117	8.8%	0.56 [0.33, 0.95]	
Subtotal (95% CI)		427		441	47.9%	0.34 [0.22, 0.53]	
Total events	135		237				
Heterogeneity: Tau ² = 0.16; Ch	ni² = 12.56,	df = 7 (F	P = 0.08);	l ² = 44	%		
Test for overall effect: Z = 4.86	6 (P < 0.000	01)					
Total (95% CI)		2338		2355	100.0%	0.58 [0.41, 0.81]	
Total events	518		618				
Heterogeneity: Tau ² = 0.28; Ch			(P < 0.000)1); l² =	71%		0.1 0.2 0.5 1 2 5 10
Test for overall effect: Z = 3.14		,					Favours [experimental] Favours [control]
Test for subgroup differences:	Chi ² = 21.2	2, df = 1	(P < 0.00	0001), I	² = 95.3%		· · · · · · · · · · · · · · · · · · ·
URE 6 Forest plots for the su	bgroup ana	lysis on	VAP: low	vs. hic	h inciden	ce of VAP.	

decrease in the incidence of VAP (38, 39), more studies include our owns have shown the decrease in the incidence of VAP after probiotic administration (10, 40, 41). The different effects may depend on the patient population and the probiotic strain studied. Many observations suggest that probiotic therapy is benefit for administration in a properly selected, critically ill population admitted to the surgical ICU (18, 42). In contrast, studies suggested that probiotics should not be administered in immunosuppressed patients or patients with severe acute pancreatitis (15, 40).

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. Our results demonstrated that compared with the control group, the probiotics group decreases bacterial colonization. The positive effects of probiotics on VAP may include (1) inducing host cell antimicrobial peptides and strengthening the gut barrier function, (2) reducing the overgrowth of potential pathogens and anti-oxidative activity, and (3) stimulating immune responses and mucus and IgA production (43). Gut flora plays a central role in the maintenance of the gut barrier and a healthy gut microbiome, probiotic therapy may be essential to optimizing outcomes in critically ill patients (44). It is worth noting that for prevention of VAP in

clinical, bundles that combine multiple prevention strategies are associated with earlier extubation and lower mortality rates, such as avoiding intubation and exposure to invasive mechanical ventilation by using high flow oxygen or non-invasive ventilation, lightening sedation, using spontaneous breathing trials to prompt early extubation, and early mobilization may be more effective (45).

In numerous clinical trials, probiotics are associated with a reduction in antibiotic-associated diarrhea (46, 47). In our study, although there was a reduction in VAP incidence, duration of mechanical ventilation, and length of ICU stay, probiotic therapy did not decrease other clinical endpoints such as ICU mortality and the occurrence of diarrhea, which are consistent with the previous metaanalysis (10). Antibiotic-associated diarrhea is characterized by the disruption of the gut microbiota altering water absorption and ultimately inducing diarrhea. One of the uses of probiotics is for the prevention of antibioticassociated diarrhea (48). However, none of the included trials reported detailed information on antibiotic use between intervention and control groups. Thus, whether probiotics alone do not cause diarrhea or probiotics do not improve antibiotic-associated diarrhea in VAP requires a more definite subgroup analysis.

	Experim	ontal	Contr	ol.		Odds Ratio	Odds Ratio
Study or Subgroup	Events				Weight	M-H, Random, 95% Cl	
Double blind	LVCIILO	Total	LVCIILS	Total	Weight		
Barraud 2010	23	87	15	80	7.3%	1.56 [0.75, 3.25]	
Giamarellos-Bourboulis 2009	15	36	16	36	6.1%	0.89 [0.35, 2.27]	
Johnstone 2021	289	1318	284	1332	10.7%	1.04 [0.86, 1.25]	- - -
Knight 2009	12	130	17	129	7.0%	0.67 [0.31, 1.47]	
Mahmoodpoor 2019	32	48	51	54	4.2%	0.12 [0.03, 0.44]	←
Morrow 2010	17	68	33	70	7.4%	0.37 [0.18, 0.77]	
Spindler-Vesel 2007	4	26	12	29	4.3%	0.26 [0.07, 0.94]	←
Subtotal (95% CI)		1713		1730	47.2%	0.62 [0.36, 1.05]	
Total events	392		428			• / •	
Heterogeneity: Tau ² = 0.34; Ch	i² = 23.39,	df = 6 (F	= 0.000 ⁻	7); l ² = ¹	74%		
Test for overall effect: Z = 1.79	,	· · ·					
No blind							
Banupriya 2015	12	70	35	72	7.1%	0.22 [0.10, 0.47]	
Forestier 2008	24	102	24	106	8.0%	1.05 [0.55, 2.00]	
Klarin 2018	7	69	10	68	5.6%	0.65 [0.23, 1.83]	
Oudhuis 2011	10	130	9	124	6.1%	1.06 [0.42, 2.72]	
Rongrungruang 2015	18	75	22	75	7.4%	0.76 [0.37, 1.57]	
Shimizu 2018	5	35	18	37	5.0%	0.18 [0.06, 0.55]	•
Tan 2011	7	26	13	26	4.9%	0.37 [0.12, 1.17]	
Zeng 2016	43	118	59	117	8.8%	0.56 [0.33, 0.95]	
Subtotal (95% CI)		625		625	52.8%	0.54 [0.35, 0.84]	
Total events	126		190				
Heterogeneity: Tau ² = 0.22; Ch			P = 0.02);	$ ^2 = 57$	%		
Test for overall effect: Z = 2.74	(P = 0.006)					
Total (95% CI)		2338		2355	100.0%	0.58 [0.41, 0.81]	◆
Total events	518		618				
Heterogeneity: Tau ² = 0.28; Ch		df = 14		01); l² =	71%		
Test for overall effect: $Z = 3.14$,, .			0.1 0.2 0.5 1 2 5 10
Test for subgroup differences:	·	,	(P = 0.71)), ² = 0	%		Favours [experimental] Favours [control]
5							
URE 7 Forest plots for the sul	haroun and	alveie on		ubla bli	nd BCTe y	e no blind BCTe	
TE / Forest plots for the sul	ogroup and	arysis of	VAF. 00	unie nili		is. NU DIINU NU IS.	

The strength of this meta-analysis includes the use of standard methods to reduce bias (comprehensive literature search, duplicate data abstraction, specific criteria for searching and analysis), and the analysis of relevant clinical outcomes in the critically ill. Additional conduct of explicit subgroup and sensitivity analysis provides evidence in the robustness of estimates.

However, there are some limitations of this meta-analysis. First, the main study limitation is the limited number of included studies and small sample size. Due to the restriction of the retrieval strategy, there was an insufficient sample to strengthen this result. Almost all of the included studies had a sample size of <100 patients, which are typically characterized as small studies. As a result, our study may be subjected to small study effect bias (49). Second, 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. The subgroup analyses based on the differences in trial design indicated that 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. Third, the diagnosis of VAP was based on various definitions in the included studies, which was a potential source of heterogeneity and may have contributed to inconsistency. In addition, it is worth noting that although no adverse effects related to probiotics were found in the included studies, it is vital to conduct safety monitoring in future clinical trials.

CONCLUSION

In conclusion, this comprehensive and updated meta-analysis analyzed the effects of probiotics in mechanical ventilated patients and found that probiotics can reduce the incidence of VAP, duration of mechanical ventilation, length of ICU stay, and bacteria colonization, but there was no significant effect on the mortality and occurrence of diarrhea. However, the significant heterogeneity and publication bias may reduce the credibility of the results. The benefit of probiotics seems clinically plausible but needed more well-designed RCTs to further validate the effect of probiotics for mechanical ventilated patients. Moreover, some new guidelines such as the updated PRISMA checklist 2020 (50), and new tools such as hierarchical nested design or competing event analyses have been recently proposed to improve the design and the analysis of future studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**,

further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HS conceived the idea, performed the analysis, and drafted the manuscript. WH and XZ contributed to the study design, data acquisition, and interpretation. JT, SZ, and XS helped in the statistical analysis. WW

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helped to frame the idea of the study and provided technical support. All the authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021. 798827/full#supplementary-material

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