EFFECTS OF PROBIOTIC PROPHYLAXIS ON THE INCIDENCE OF VENTILATOR - ASSOCIATED PNEUMONIA AMONG CRITICALLY ILL PEDIATRIC PATIENTS: A META-ANALYSIS

CAROLYN GRACE C. TONGSON, MD, MARIA EVA I. JOPSON, MD

ABSTRACT

BACKGROUND: Among critically ill pediatric patients, a common complication experienced is nosocomial pneumonia. One field that garnered special interests as an alternative and promising way of preventing infection is the utilization of Probiotics. But whether it can prevent occurrence of ventilator-associated pneumonia (VAP) among critically ill pediatric patients remains unclear

OBJECTIVES: To determine whether probiotic supplementation will prevent the incidence of ventilator-associated pneumonia among critically ill pediatric patients.

METHODS: Literature search was conducted in PubMed, MEDLINE, EMBASE, CINAHL, SciHub, Herdin, Google Scholar, the Cochrane Central Register of Controlled Trials, and the Cochrane Database of Systematic Reviews to identify all relevant randomized controlled trials (RCTs) published between 1980 and 2016. The reviewers extracted data and reviewed the quality of the studies independently.

RESULTS: Three randomized controlled studies with a total of 327 pediatric patients admitted at the PICU were analyzed. Pooled analysis showed a statistically significant reduction in nosocomial pneumonia rates (odd ratio [OR] = 0.31, 95% CI 0.18 to 0.55, $P< 0.0001$, $I^2 = 53\%$) and statistically significant difference was found regarding overall mortality (OR =0.51 , 95% CI 0.30 to 0.88, $P = 0.01$, $I^2 = 0\%$) due to probiotics. However, no statistically significant difference was found between groups regarding duration of stay in the PICU (Mean Difference [MD] in days = 2.93, 95% CI 1.84 to 4.01, $P < 0.00001$, $I^2 = 97\%$), and duration of stay in the hospital (MD = 4.33 days, 95% CI 2.85 to 5.81, $P <$ .00001, $I^2 = 97\%$).

CONCLUSION AND RECOMMENDATIONS: The use of probiotics was associated with statistically significant reduction in the incidence of VAP in critically ill children. However, larger and well-designed, multi-center, RCTs are needed to further establish the effects of probiotic in the pediatric population of critically ill children who are at risk of developing nosocomial infection.

KEYWORDS: Probiotics, Ventilator Associated Pneumonia, Pediatric Intensive Care Unit

INTRODUCTION

Despite progress in public health and hospital care, nosocomial infection is a major public health concern in both developing and developed countries. An increased risk in morbidity and mortality has been seen among in-patients inflicted with nosocomial infection. They are caused by a wide range of pathogens, and common sites of infection are said to be the bloodstream, respiratory tract, and urinary tract. The World Health Organization (WHO) showed that, at any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospitals. The highest frequencies were reported to have a prevalence of 11.8% and 10.0% respectively in the Eastern Mediterranean and South-East Asian Regions.

Among critically ill patients, a common complication experienced is nosocomial pneumonia, specifically in patients who are intubated for more than 48 hours. This renders nosocomial pneumonia to be responsible for significant in-hospital morbidity and mortality. When mechanically ventilated patients develop nosocomial pneumonia, it is then termed ventilator associated pneumonia (VAP). Given the condition of these patients, multiple risk factors were identified and thought to increase

bacterial colonization of the aero-digestive tract and facilitate the entry of pathogenic bacteria into the lower respiratory tract.

Recent findings suggest that frequent use of antibiotics may lead to the emergence of multidrug-resistant organisms, or may lead to the depletion of good microorganisms, and thereby just putting children more at risk to infection. In effect, it is this endemicity and persistence in resistant strains from widespread use of antimicrobials that facilitate bacterial infection spread in the pediatric health care setting. Many strains that were once susceptible to antimicrobials are now rendered resistant to treatment. And this is a problem particularly in developing countries such as the Philippines where more expensive second-line antibiotics may not be easily available or affordable for the Filipino families.

This recent trend on resistance to antimicrobials pushed for the creation of improved surveillance and implementation of more effective preventive interventions. One field that garnered special interests as an alternative and promising way of preventing infection is the utilization of Probiotics. Lately, the use of harmless bacteria through Probiotics to displace pathogenic strains has gained much attention in addressing different infections, including hospital-acquired ones. However, whether probiotics can prevent occurrence of nosocomial pneumonia among admitted critically-ill pediatric patients is still unclear. Therefore, there is a need to determine if children admitted at the pediatric intensive care unit and supplemented with probiotics will have better health outcomes in terms of development of nosocomial pneumonia during their hospital stay.

As a result of existing studies demonstrating probiotics' potential in up regulating immune defenses and reducing bacterial translocation, there has been a rapidly growing interest in the clinical application of probiotics. A few clinical trials have already begun to look at the incidence of infections with probiotic use and have demonstrated promising results. However, current evidence and opinions suggest that data to conclusively determine whether probiotics can be safely used in prevention of nosocomial infections, particularly reduction of incidence of ventilator-associated pneumonia is still insufficient.

Although there is theoretical plausibility shown by current literature in the use of probiotics for infection prevention, most of which are inconsistent in results and utilized different sets of population. In addition, a bench research is yet to be performed to determine the most appropriate probiotic formulation for various clinical applications as specific strains are thought to be effective in certain disease states.

In this respect, this study was conducted to contribute to the knowledge of whether probiotics can be used in the prevention of nosocomial infection, particularly of nosocomial pneumonia, in critically ill children admitted in pediatric intensive care units. The study intended to re-evaluate the present knowledge or hypothesis that administration of probiotics in critically ill children may reduce the incidence of ventilator-associated pneumonia. In this light, it is hoped that a promising alternative may be used to lessen the incidence of nosocomial pneumonia, thereby decreasing hospital stay, preventing morbidities and mortalities, reducing cost needed for treatment, lessening side effects common to antibiotic use, and combating antibiotic resistance.

This study aimed to serve as a guide in deciding whether to supplement probiotics among pediatric patients admitted at PICU of any Pediatric Hospital and needing antibiotic therapy. In addition, it is possible that there are other hospitals/health care institutions that are having the same dilemma regarding ventilator-associated pneumonia and antibiotic resistance. This study can serve as a blueprint on how to manage such issues with new potential alternatives.

METHODOLOGY

To identify studies for inclusion in this review, literature search was conducted in PubMed, MEDLINE, EMBASE, CINAHL, SciHub, Herdin, Google Scholar, the Cochrane Central Register of Controlled Trials and the Cochrane Database of Systematic Reviews to identify all relevant randomized controlled trials (RCTs) published between 1980 and April 2016.

The search was limited to studies conducted in humans. The literature search used search terms containing "randomized", "clinical trial", "probiotics", "nosocomial infection", "health care associated pneumonia", "ventilator associated pneumonia", "pediatrics", "intensive care". No language and publication restrictions were applied. Personal files, reference lists of relevant review articles, and proceedings of major relevant conferences were also be reviewed as secondary searches. Excluded trials included those with no specific disease being studied, non- RCTs, and trials on animals other than humans.

The author used titles and abstracts to already exclude trials which clearly did not meet the set inclusion criteria. The common reasons encountered for exclusion of the articles from the electronic search were non-human studies, the non-use of probiotics, measurement of outcomes other than incidence of VAP. Full articles were retrieved for further assessment if the abstracts indicated that there was a possibility that the study fulfilled the inclusion criteria. The journals were screened, and peer reviewed by another reviewer to assess study eligibility. Analysis was restricted to double-blind, randomized controlled trials (RCTs). For this meta-analysis, those RCTs that compared administration of probiotic vs. placebo in critically ill patients, and that reported the incidence of VAP, were considered.

The investigator and another peer reviewer independently reviewed and assessed inclusion criteria and quality of trials. Three potentially eligible papers were identified and reviewed.

Data concerning details of study population, intervention and outcomes were extracted independently by the reviewers using a specifically designed data extraction form of "The Cochrane Collaboration" (Cochrane Library). From each paper, the researcher extracted information related to

- General Information: published/unpublished, title, authors, year of publication, number of patients
- Trial Characteristics: method of randomization and allocation concealment, blinding (participants, clinician, outcome assessor, loss of participants to follow up, intention to treat analysis)
- Intervention: doses, frequency of probiotic supplementation
- Participants characteristics: inclusion and exclusion criteria, age group, number of patients in each intervention
- Outcomes: the primary outcome was the incidence of patients that developed ventilator-associated pneumonia (VAP) following probiotic/control. The researcher used the authors' definition of NP or VAP if they included clinical, microbiologic and radiologic criteria. Secondary outcomes were mortality, length of stay in the ICU and in hospital, and reports of adverse outcomes.
- Results: continuous data were expressed as weighted mean differences and standard deviation, use of intention to treat a analysis.

Differences in data extraction were resolved by discussion and consensus. When necessary, additional information was sought from the authors of the studies.

Each included study was assessed based on the following indicators of risk of bias as listed below. A verdict of LOW RISK meant low risk of bias, a HIGH RISK meant high risk of bias; and UNCLEAR RISK for unknown risk of bias—were used as the criteria for judging risk.

- Adequate sequence generation
- Allocation concealment
- Blinding of participants, personnel, and assessors
- Incomplete outcome data
- Selective outcome reporting

Meta-analysis was conducted using Review Manager 5.3 (Cochrane Collaboration, UK). A fixed-effects method (Mantel-Haenszel method) was used. The author computed pooled odds ratios (ORs) and 95% confidence intervals (CIs) from adjusted ORs and 95% CIs reported in the observational studies.

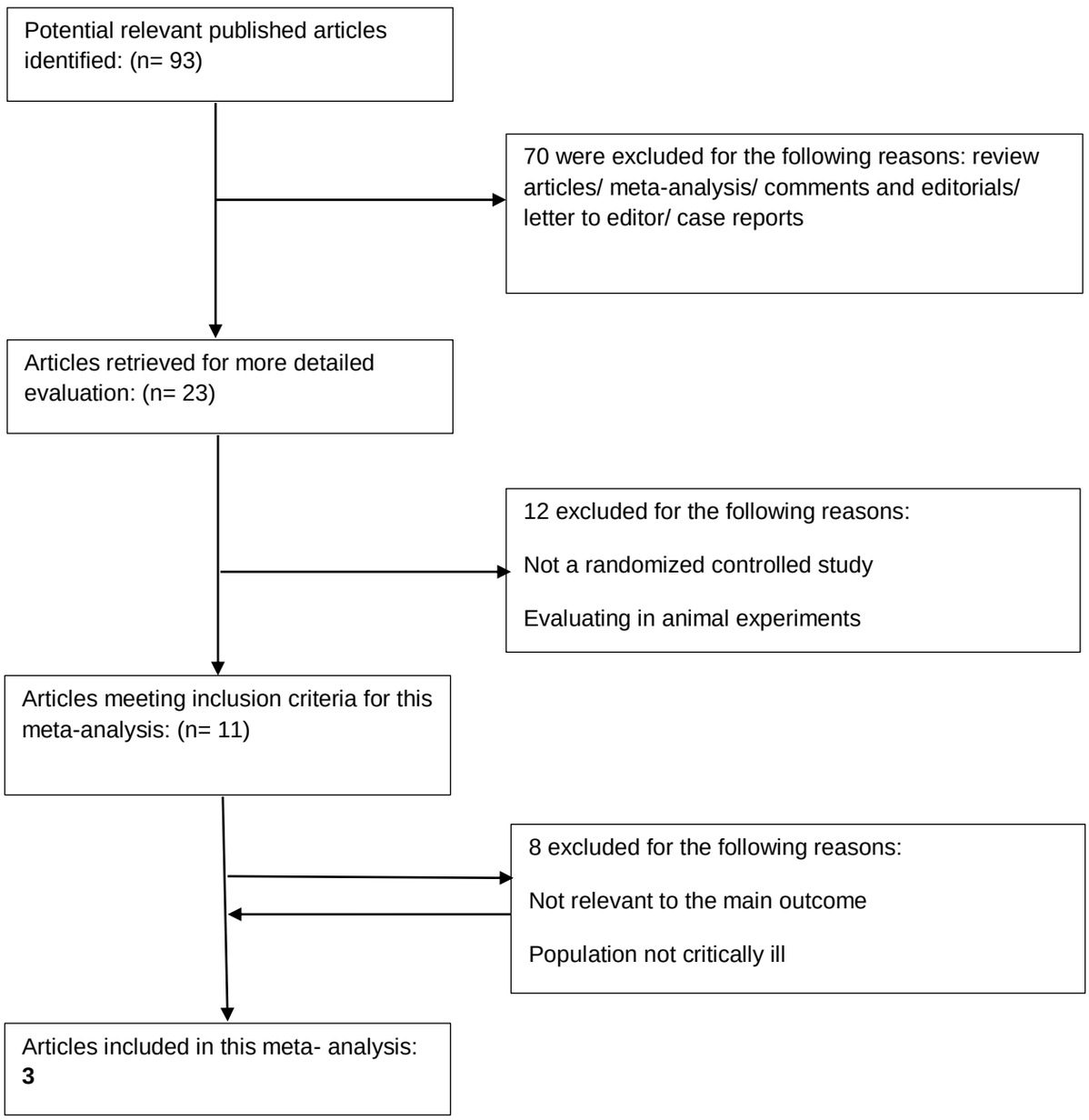
If the researcher was unable to extract all the information with regards to the details of

the study, the plan was to contact the authors. Fortunately, all numerical results in the published reports were adequate to proceed with the study. Quantification of the effect of heterogeneity was assessed by means of I^2. We predefined heterogeneity as low, moderate or high with I^2 values > 25%, 50%, and 75%, respectively. In the analysis of heterogeneity, we considered a *P-value* < 0.10 statistically significant. Both positive and negative results were reported among the studies. Publication bias was assessed by a funnel plot using the occurrence of VAP as an endpoint.

RESULTS

Our search retrieved a total of 131 references. After screening them against the inclusion criteria. Three studies were included in this meta-analysis. A flowchart diagram for the studies evaluated and the reasons for exclusion are shown in **Figure 1.**

Figure 1. Flow Chart of Study Selection. Pooled ORs were calculated using Mantel-Hanszel (M-H) Estimator. Study-level data were pooled using fixed-effects model.



Characteristics of the included studies are summarized in **Table 1**. A total of 332 critically ill patients were included in these three studies. The trials were carried out at a single center or hospital. All three trials recruited patients in pediatric intensive care units (PICU), both medical and surgical cases included. The patients recruited were those needing mechanical ventilation as deemed necessary by the expert clinicians. Two studies excluded patients with evidence of perforated intestine, mechanical GI obstruction, ANC < $0.5x\ 10^9$, admitted at PICU >72 hours, used probiotics in the week before study accession, and if there was lack of parental presence/consent.

Table 1. Characteristics of the study population in various studies

Study, Year	**Study Design**	**Population**	**Disease Severity Score**	**Regimen Used**	**Route of Administration/ Duration of Intake**
Banupriya *et al.*, 2014 [24]	Open-Label Randomized Controlled Trial (RCT)	All children aged 12 years or less admitted to PICU and who were likely to need mechanical ventilation for more than 48 hrs were recruited	Pediatric Risk of Mortality (PRISM III) Score 11.61 ± 5.63 vs 11.25 ± 6.58	Probiotic capsules containing 2 billion CFU of Lactobacillus, 1 billion CFU of Bifidobacterium, and 300 million CFU of Streptococcus thermophilus were used in this study. One capsule was administered twice a day mixed with milk (or 5 ml of 5 % dextrose solution if enteral feeding had not been started).	Given through a nasogastric tube. A total of 6.6 billion CFU of probiotic organisms per day was administered to each child in the probiotic group for the initial 7 days or till discharge, whichever was earlier.
El-Wakeel *et al.*, 2016 [25]	Double Blinded Randomized Placebo-Controlled Trial (DBRCT)	Patients admitted at the Pediatric Intensive Care Unit (PICU) of a University's Childrens' Hospital requiring MV > 48 hrs were said to be eligible	Pediatric Risk of Mortality (PRISM) Score 33.9±13.9 vs 34.2±15.6	One Lactobacillus rhamnosus strain GG capsule once a day (Culturelle, 10×10^9 cells/capsule, ConAgra Foods, Omaha, NE) was used. Probiotic capsules were prepared in a suspension of (5 ml) of 5% dextrose.	Administered by orogastric, nasogastric tube or by mouth in patients who could be fed orally for the duration of hospitalization.
Honeycutt *et al.*, 2007 [9]	Double Blinded Randomized Placebo-Controlled Trial (DBRCT)	Children admitted at the medical-surgical PICU of a university-affiliated hospital requiring MV > 48 hours	N/A	One capsule of Lactobacillus rhamnosus strain GG (Culturelle, $10x10^9$ cells/ capsule, ConAgra Foods, Omaha, NE) once a day. The probiotic and placebo capsules were prepared in a suspension of 5 mL of 5% dextrose. An appropriate normal saline flush was administered in patients with an orogastric/naso-gastric tube.	Administered by mouth in those able to orally feed or by orogastric/nasogastric tube. Patients continued the protocol until discharge from the hospital, parental request to withdraw from the study, or the death of the patient.

The quality assessment of these studies were summarized in a table that can be found in the appendix section as table 3. Results of the meta-analyses that explored the effects of probiotic prophylaxis in the primary and secondary clinical outcomes are shown in **Table 2.**

Table 2. Outcome data of all randomized controlled trials included in the meta-analysis (comparison of probiotic versus control)

Study	Incidence of VAP (n/N)		Length of ICU Stay, median days (range)		Length of Hospital Stay, median days (range)		ICU Mortality (n/N)	
	Placebo	*Probiotic*	*Placebo*	*Probiotic*	*Placebo*	*Probiotic*	*Placebo*	*Probiotic*
Banupriya *et al.*,* 2014 [24]	35/73	12/73	12.54 ± 9.91	7.7± 4.6	19.17 ± 13.51	13.13 ± 7.1	23/73	17/73
El-Wakeel *et al.*, 2016 [25]	15/50	7/75	15.6 ± 11.6	14.8 ± 11.8	N/A	N/A	15/50	10/75
Honeycutt *et al.*, 2007 [9]	0/30	2/31	7 ± 2.5	12.2 ± 2.5	11.1 ± 3.3	17.6 ± 3.2	4/30	2/31

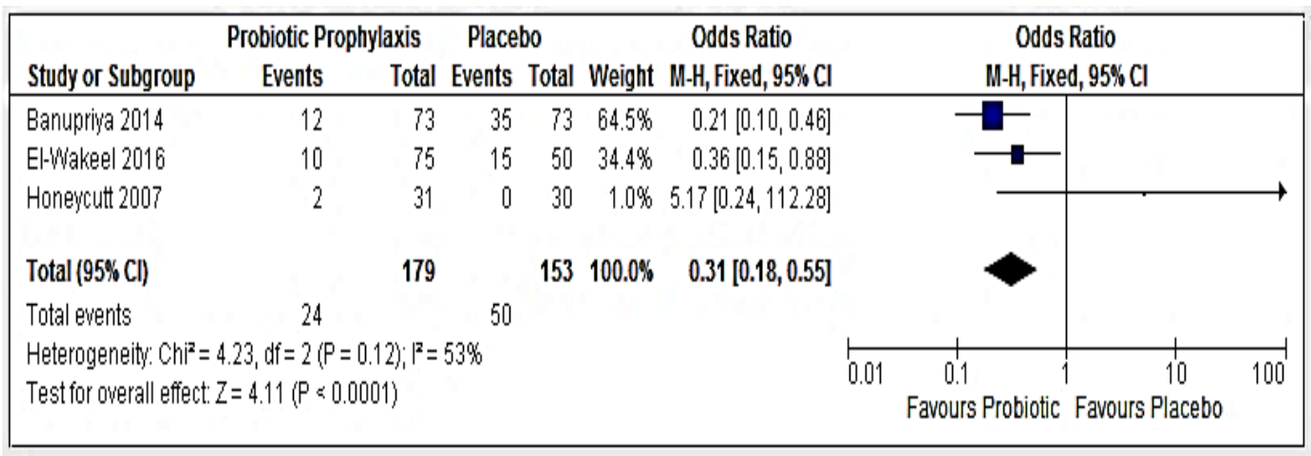
ICU: intensive care unit; NA: not available; VAP: ventilator-associated pneumonia

***** The control group did not receive any placebo

A. Nosocomial Pneumonia and Subgroup Analyses

Results from the three trials (332 patients) were available to examine the effects of oral probiotics on the incidence of VAP [9,24,25]. A moderate level of heterogeneity was found among the identified comparisons (I^2 = 53%, P = 0.12). Pooled analysis showed that the use of probiotics was associated with a statistically significant reduction in the incidence of NP in critically ill patients (OR = 0.31, 95% CI 0.18 to 0.55, $P<$ 0.0001) (**Figure 2**)

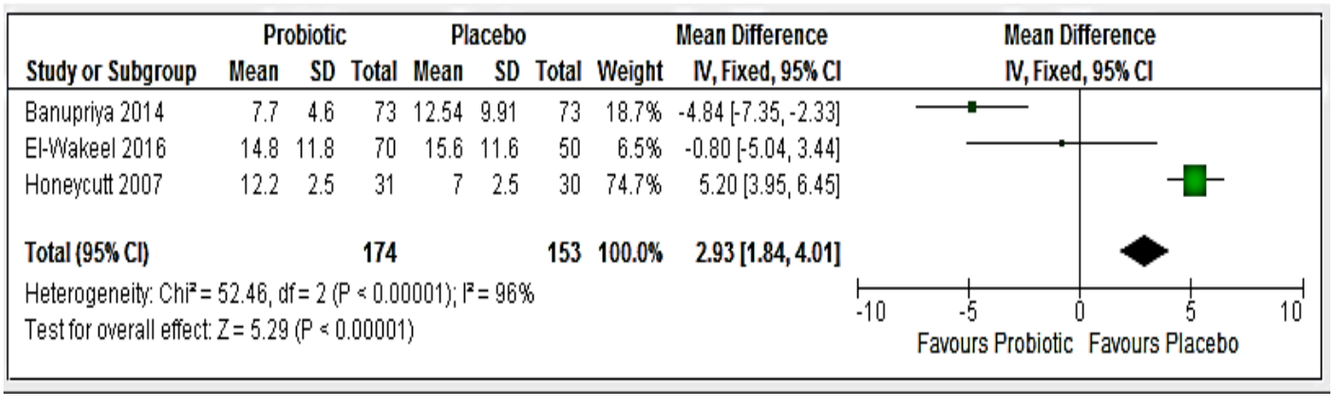
Figure 2. Forest plot showing the effect of probiotics on the occurrence of ventilator associated pneumonia (VAP) in critical ill patients



B. Duration of Stay in the Intensive Care Unit

Data from the three studies were included in the analysis of the duration of stay in the intensive care unit [9,24,25]. There was significant heterogeneity in the length of PICU stays (I^2 = 96%, P = <0.00001) (**Figure 3).** There was significant difference between the compared groups regarding this outcome (MD in days = 2.93, 95% CI 1.84 to 4.01, *P < 0.00001).*

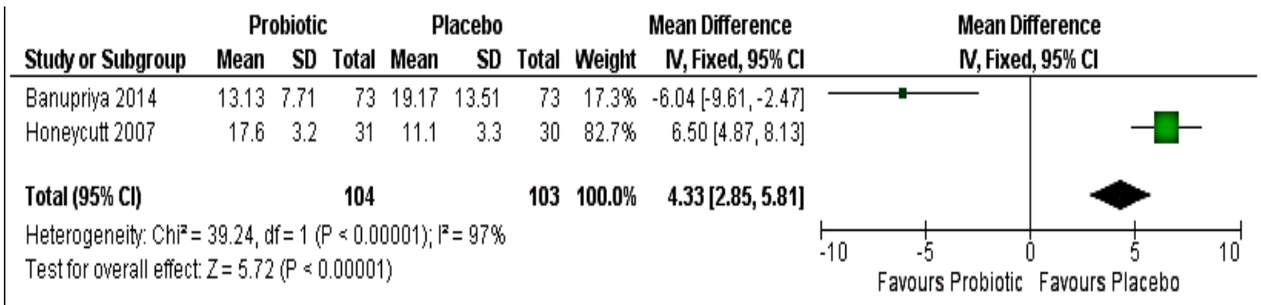
Figure 3. Forest plot showing the effect of probiotics on length of ICU stay (in days). Mean differenceswere estimated by the inverse variance (IV) approach.



C. Duration of Stay in the Hospital

Two of the studies were included in the analysis of the length of stay in the hospital [9,24]. Again, there was a high level of heterogeneity in the length of hospital stay found between this comparison (I^2 = 97%, P = <0.00001) There was apparent effect of probiotics therapy on the duration of stay in the hospital, with a mean difference (MD) of 4.33 days (95% CI 2.85 to 5.81, P < .00001) (**Figure 4).**

Figure 4. Forest plot showing the effect of probiotics on length of hospital stay (in days). Mean differences were estimated by the inverse variance (IV) approach.



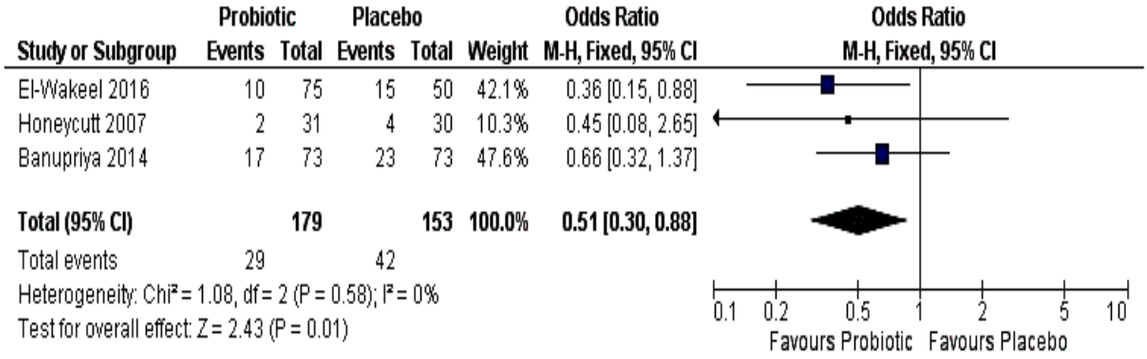
Caption

Forest plot of comparison: 1 Probiotic Prophylaxis versus Placebo, outcome: 1.3 Length of Hospitalization (days).

D. Overall Mortality

Results of all three trials were available for the analysis of mortality during the entire ICU stay [9, 24, 25]. A meta-analysis of these trials found that probiotic administration had an effect on overall mortality during the hospital stay or had a significant difference in ICU mortality between probiotics group and placebo group (OR =0.51 , 95% CI 0.30 to 0.88, P = 0.01) (**Figure 5).** There was no heterogeneity between trials (I^2 = 0%).

Figure 5. Forest plot showing the effect of probiotics on ICU mortality. Pooled ORs were calculated using the Mantel-Haenszel (M-H) Estimator.



Caption

Forest plot of comparison: 1 Probiotic Prophylaxis versus Placebo, outcome: 1.4 Mortality.

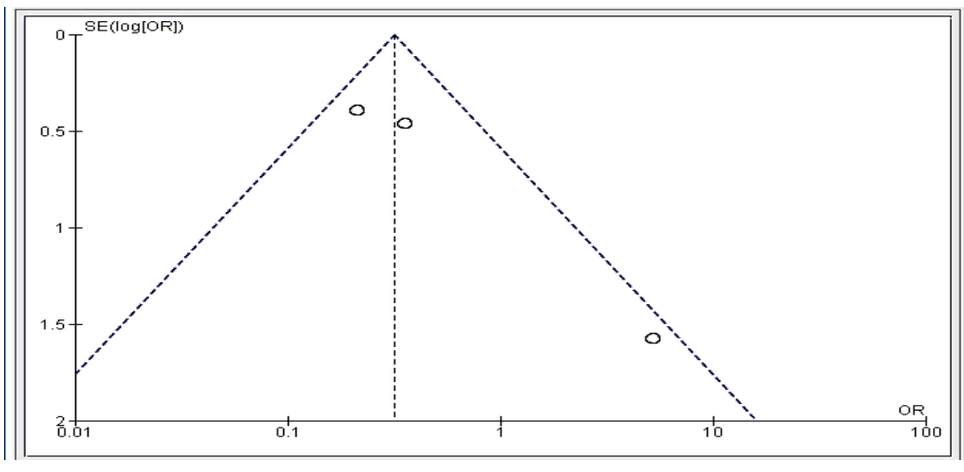
E. Adverse Events

For the study done by Banupryia *et al.*, the probiotics were administered for the initial 7 days or until discharge, whichever was said to be earlier. While for the study of El Wakeel *et al.*, the probiotics were administered for the duration of the hospitalization. For these two studies, there were no adverse events such as cases of bacteremia caused by *Lactobacillus* were observed in the patients of the intervention group. On the other hand, in the study of Honeycutt *et al.*, the probiotics were given during the hospitalization until they are discharged, withdrew from the study, or died. In the study period, there were no cases of L. bacteremia in the study population reported and no known serious adverse effects occurred in any subject. However, because of recent safety concerns regarding the administration of Lactobacillus GG in critically ill pediatric patients and a lack of benefit in their analysis, the study was eventually terminated by the study investigators.

F. Publication Bias

Upon visualization of the funnel plot for the primary outcome, there is a possibility of publication bias (absence of small studies, shown in the left lower corner of **Figure 6.** But this may also be attributed to the small number of studies included in the meta-analysis. As well as presence of heterogeneity in the correlation between study size and intervention effects.

Figure 6. Funnel plot showing possibility of a small publication bias. SE, standard error: OR, odds ratio.



DISCUSSION

The meta-analysis found that probiotics administration was associated with statistically significant reduction in the incidence of ventilator-associated pneumonia among critically-ill patients. The pooled results also showed that probiotics affect the other secondary endpoints of the study (ie. overall mortality, length of stay in the hospital, and length of stay in the ICU). However, due to a large heterogeneity in the length of hospital stay and ICU stay, the effects found in these sub-studies may be non-comparable that one cannot confidently say that the combined estimate will be a meaningful description of the outcome produced.

The current meta-analysis is different from previous reviews in several aspects. One, it includes more eligible studies than previous reviews on probiotics administration that recruited only selected populations (ie. surgical patients only).[26] Trials done before were mostly that of critically adult patients [27], while this study focused on critically ill pediatric patients, and therefore results are more applicable across clinical situations encountered with critically ill children.

In order to diminish the number of confounding factors, the study also excluded studies that used interventions known to be effective in preventing nosocomial pneumonia, namely the use of chlorhexidine and antibiotic decontamination of the digestive tract as control groups [28,29].

The results of this study appear like previous studies done by Siempos *et al.* [30] and Pitsouni *et al.* [26], but inconsistent with the results of the systematic review by Watkinson *et al.* [27]. Siempos *et al*. found that administration of probiotics was beneficial in the incidence of both VAP and NP, length of stay in the ICU and colonization rates of *Pseudomonas aeruginosa* in the respiratory tract. The reasons for inconsistent results, even in this study, may be partly due to differences in focused clinical outcomes.

The results of this meta-analysis should be interpreted with caution based on other considerations. As the diagnosis of pneumonia may be more subject to bias due to it being a more subjective outcome as compared to mortality or length of ICU stay. And this may in part explain the marked reduction in pneumonia found in these studies. In addition, the presence of an effect on secondary outcomes, but that of presence of high heterogeneity across studies, may be from the small number of pooled RCTs and small number of total patients. Lastly, the treatment duration in some studies were likely too short to demonstrate maximum benefits. Consequently, it may be difficult to derive conclusive results based on this meta-analysis due to the lack of standard protocols and insufficient number of included patients.

Our analysis has several limitations. First, as mentioned above, there is presence of heterogeneity among the study categories and variables- both clinical and statistical, that were used for establishing NP or VAP. Some factors that may not be comparable in the trial might have affected the clinical outcomes derived. The moderate level of heterogeneity seen from the comparison on the incidence of ventilator associated pneumonia may be attributed to differences among the local hospitals' PICU set-up, their practices, and the way the probiotics were administered. These differences may explain the statistical heterogeneity in some of the secondary outcomes investigated. Second, although we have pooled similar data across all trials, the number of participants per trial may be not sufficient to exclude significant clinical benefit. Thirdly, the setting of most trials was done only in single hospital centers and may have inherent bias related to their local practice habits. Finally, a possibility of publication bias based on funnel plot may discount our extensive search for relevant studies using multiple search items and removing language restriction. Finally, although initial results seem to be promising for the use of probiotics to prevent nosocomial pneumonia, there is still insufficient evidence to conclude to pediatricians that administration of probiotic prophylaxis is associated with lower incidence of VAP in critically ill patients. In addition, studies that investigate which particular probiotic strain has more benefit including superiority in terms of dose, preparation, duration, safety, and route, are still lacking. These are areas that are yet to be ventured when it comes to evaluation of probiotics use in large-scale randomized controlled trials for nosocomial pneumonia.

CONCLUSIONS AND RECOMMENDATIONS

The use of probiotics was associated with statistically significant reduction in the incidence of Ventilator-Associated Pneumonia in critically ill children. However, there is lacking evidence to support claims of beneficial effects on other clinically important outcomes such as length of hospital or ICU stay. Larger and well-designed, multi-center, RCTs are needed to establish the effects of probiotic prophylaxis in the pediatric population of critically ill children who are at risk of developing nosocomial infection.

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