 doi:10.20944/preprints201810.0002.v1

*Review*

# Role of probiotics to prevent and reduce the duration of upper respiratory infections in ambulatory children: Systematic Review with Network-Meta Analysis

Pedro Gutierrez-Castrellon[1,*] MD, MSc, DSc; Zvi Weizman[2] MD; Silvia Cruchet[3] MD, MSc.; Ener Cagri Dinleyci[4] MD; Carlos Jiménez-Gutiérrez[5] MPH, DPH & Gabriel López-Velazquez[6,*] Biologist, PhD

[1]Centro de Investigación Translacional en Salud Materno-Infantil. Hospital General Dr. Manuel Gea Gonzalez. Ministry of Health. Mexico; Latin-American Society for Pediatric Gastroenterology, Hepatology and Nutrition (LASPGHAN) & Research Division-Health Sciences. Universidad Tecnológica de México-UNITEC; [2]Instituto de Nutrición y Tecnologia de los Alimentos. Universidad de Chile. Santiago de Chile & Latin-American Society for Pediatric Gastroenterology, Hepatology and Nutrition (LASPGHAN); [3]Pediatric Gastroenterology and Nutrition Unit, Soroka Medical Center, Faculty of Health Sciences, Ben- Gurion University, Beer-Sheva, Israel: [4]Eskisehir Osmangazi University Faculty of Medicine. Department of Pediatrics. Eskisehir Turkey TR-26480; [5]Center for Analysis of Evidence in Health & Cochrane Affiliate. Hospital General Dr. Manuel Gea Gonzalez. Ministry of Health. Mexico;[6]Biomolecules and Infant Health-EIMYT Lab. Instituto Nacional de Pediatría. Ministry of Health. Mexico

* Correspondence:

Pedro Gutierrez-Castrellon MD, MSc, DSc. Centro de Investigación Translacional en Salud Materno-Infantil. Hospital General Dr. Manuel Gea Gonzalez. Ministry of Health. Mexico Calz. Tlalpan 4800, Sección XVI, 14080, Tlalpan, Mexico city. Mexico. +5255-4000-3000. Ext. 1246. inpcochrane@gmail.com

Gabriel López-Velázquez Biologist, PhD. Biomolecules and Infant Health-EIMYT Lab. Instituto Nacional de Pediatría. Ministry of Health. Mexico Insurgentes sur 3700-C, Cuicuilco, 04530, Coyoacan, Mexico city. Mexico. +5255-1084-0900. glv_1999@yahoo.com

**Abstract:** Background. Upper respiratory infections (URIs) remains as significant cause of morbidity in children. Evidence on efficacy of probiotics to prevent URIs in children is increasing. This systematic review was assembled to analyze evidence about the efficacy of probiotics to reduce duration of upper respiratory infections in ambulatory children. Methods. Randomized controlled trials (RCTs) comparing probiotics vs. placebo to prevent URIs, published between 2001 and 2016 were considered. Quality evaluation was evaluated using CONSORT. Standard mean difference (SMD) or risk ratio (RR) was calculated. Network Meta-Analysis (NMA), using a random effect model was assembled. Results. 31 RCTs were evaluated and 20 studies were included with 3,635 children randomized to probiotics and

3,433 to placebo. *Lactobacillus reuteri* [SMD -0.56 $CI_{95\%}$ (-0.72 to -0.41), p 0.0001] and *Lactobacillus acidophillus* [SMD -0.33 $CI_{95\%}$ (-0.60 to -0.06), p 0.01] were superior to placebo to reduce duration of URIs. *L. rhamnosus* GG showed tendency [SMD -0.14 $CI_{95\%}$ (-0.28 to 0.0), p 0.048]. On the network forest plot *L. reuteri* showed preventive equivalence when was compared to L. rhamnosus GG, L. casei and BB12. Conclusions. *Lactobacillus reuteri*, *Lactobacillus rhamnosus* GG and *Bifidobacterium BB12* are evidence-based alternatives to be considered to prevent URIs in children.

**Keywords:** Probiotics, Upper Respiratory Infections, Network Meta-Analysis

---

**1. Introduction**

URIs are a common problem in the first decade of life. The yearly prevalence of respiratory tract infections in an otherwise healthy 3-year old child is about three to 10 infections**.** (1-4) Although most respiratory tract infections are generally self-limiting and in more than 70% are from viral etiology, the use of antibiotics around the world is very high. Over a time period of 12 months, between 30% to 75% of all children and adolescents younger than 15 years of age takes antibiotics for URIs. (5) About 85–88 % of all respiratory infections in children are URIs with pharyngitis/tonsillitis as the most frequent URIs. (6-8) This type of infections imposes a significant economic burden on health systems and individual families in developing countries. We recently estimated that among children aged < 5 years, the median direct cost of ARI was US$135 in private and US$54 in public institutions. (9) Considering that in the most of cases URIs in ambulatory children are from viral etiology, that there is no consensus to start treatment of URIs with antibiotics, taking into account all the antibiotic associated adverse events (10), and secondary to the effects on immunity and inflammation previously proved for some probiotics, there has been a growing interest in the use of this alternatives for treatment and prevention of this type of infections in children and adults. (11-22) The first randomized controlled trial (RCT) about the use of probiotics to prevent URIs in outpatient children was published in 2001. It was conducted in 18 daycare centers in Helsinki, Finland. 571 healthy children aged 1 to 6 years were included (n=282 in probiotic group and 289 in control group). *Lactobacillus rhamnosus* GG was administrated in milk. Children in the Lactobacillus group had fewer days of absence from day care because of illness [4.9 (95% confidence interval 4.4 to 5.5) vs 5.8 (5.3 to 6.4) days, 16% difference, P = 0.03. There was also a relative reduction of 17% in the number of children suffering from respiratory infections with complications and a 19% relative reduction in antibiotic treatments for respiratory infection [unadjusted absolute % reduction - 9.6 (- 18.2 to - 1.0), P = 0.03; adjusted odds ratio 0.72 (0.50 to 1.03), P = 0.08] in the Lactobacillus group. (11) Twelve years later the first meta-analysis was published. Four RCTs involving 1805 participants were considered. Authors concluded that compared with placebo, probiotics administration was associated with a reduced incidence of acute otitis media (four RCTs, n=1805, RR 0.76, 95% CI 0.64-0.91, fixed effects model, NNT 17, 95% CI 11-46), a reduced risk of URIs (one RCT, n=281, RR 0.62, 95% CI 0.50-0.78, NNT 4, 95% CI 3-8) and antibiotic treatments (four RCTs, n=1805, RR 0.80, 95% CI 0.71-0.91, fixed effects model). Unfortunately, the number of RCTs that supported the reduction of risk for URIs was only

 doi:10.20944/preprints201810.0002.v1

one, establishing the need to assembly other RCTs. (12) In 2014 and 2015 were published others meta-analysis on the same topic, including 10 and 12 RCTs respectively. (13,14) Unfortunately, the included studies in children and adults and with specific populations (i.e. malnourished patients), what made recommendations difficult to extrapolate to healthy children. In 2016, a fourth meta-analysis was published, which included only RCTs performed in children. A total of 23 trials involving 6269 children were eligible. None of the trials showed a high risk of bias. The quality of the evidence of outcomes was moderate. The age range of subjects was from newborn to 18 years. The results of meta-analysis showed that probiotic consumption significantly decreased the number of subjects having at least one URI episode (17 RCTs, 4513 children, relative risk 0.89, 95% CI 0.82–0.96, P=0.004). Children supplemented with probiotics had fewer numbers of days of RTIs per person compared with children who had taken a placebo (6 RCTs, 2067 children, MD 0.16, 95% CI 0.29 to 0.02, P=0.03), and had fewer numbers of days absent from day care/school (8 RCTs, 1499 children, MD 0.94, 95% CI 1.72 to 0.15, P=0.02). However, there was no statistically significant difference of illness episode duration between probiotic intervention group and placebo group (9 RCTs, 2817 children, MD 0.60, 95% CI 1.49 to 0.30, P=0.19). (15) Unfortunately, this meta-analysis included not only RCTs focused on prevention, but also RCTs assembled for treatment of respiratory infections. Finally, a Network Meta-Analysis (NMA) was published recently. It was focused on the effects of probiotics to prevent respiratory infections in children and adolescents. Twenty-one trials with 6.603 participants were included. Pairwise meta-analysis suggested that Lactobacillus casei rhamnosus (LCA) was the only effective probiotic to the rate of RTIs compared to placebo (RR0.38; CI 0.19–0.45). NMA showed that the LCA exhibited 54.7% probability of being classified in first, while the probability of Lactobacillus fermentum CECT5716 (LFC) being last in the ranking was 15.3%. LCA showed no better effect compared to other probiotic strains by indirect analysis. (16) When this paper was reviewed by our group we found some papers assembled with different objectives (i.e. to reduce the risk of necrotizing enterocolitis and/or neonatal sepsis in preterm (17); to reduce the cases of recurrent otitis media (18,19); to prevent nosocomial infections (20,21) or to reduce the presence of bocaviruses in mouth (22)]. Considering limitations of previous reviews, we decide to assembly this new systematic review with NMA limited to evaluate the strain specificity of *Lactobacillus reuteri* and other probiotics to prevent and reduce the occurrence and duration of upper respiratory infections in ambulatory children.

## 2. Materials and Methods

Systematic review and network meta-analysis was assembled according to PRISMA statements (23), incorporating Network Meta-analyses of Health Care Interventions (24), and approved by the Research Committee at Hospital General Dr. Manuel Gea Gonzalez, Ministry of Health. Mexico. (Supplementary materials)

*Search strategy*

We included in this systematic review only double-blind, randomized, controlled clinical trials (RTCs), published between January 2001 and January 2016, in English or Spanish language. An exhaustive search was conducted in Medline, Embase, Cumulative Index to Nursing and Allied Health (CINAHL), PsycINFO, the Cochrane Central Register of

 doi:10.20944/preprints201810.0002.v1

Controlled Trials, Lilacs, Artemisa and in the databases of the principal international regulatory agencies in order to identify relevant studies published. PubMed searching algorithms was ((“infant” [MeSH Terms] OR “infant” [All Fields]) OR (“preschool child” [MeSH Terms] OR “preschool child” [All Fields]) OR (“child” [MeSH Terms] OR “child” [All Fields])) AND ((“upper respiratory infection” [MeSH Terms] OR “upper respiratory infection” [All Fields]) OR (“upper respiratory infections” [MeSH Terms] OR “upper respiratory infections” [All Fields]) OR (“upper respiratory tract infection” [MeSH Terms] OR “upper respiratory tract infection” [All Fields]) OR (“upper respiratory tract infections” [MeSH Terms] OR “upper respiratory tract infections” [All Fields])) AND ((“probiotics” [MeSH Terms] OR “probiotics” AND (Clinical Trial[ptyp] AND (“2001/01/01” [PDAT]: “2016/01/31” [PDAT]) AND “humans” [MeSH Terms] AND (English[lang] OR Spanish[lang])).

*Study selection and outcome measures*

RCTs that compared the use of probiotics (mono-strain or combined strains) versus placebo or active treatment in ambulatory or attending daycare centers infant (birth to 23 months of age), preschool child (2-5 years old) or child (6 to 12 years old) were consider to be included in this network meta-analysis. All different probiotics used to prevent and/or reduce the occurrence of URIs were included in 14 nodes of intervention (Figure 1). Primary outcome analyzed was the reduction of the duration of the episodes of URIs observed during intervention. Secondary outcomes were the reduction of a) at least one episode of URIs; b) the number of days with URIs; c) the use of antibiotics and d) the days of scholar absentees.

*Data extraction and quality analysis*

Quality evaluation of studies was performed in pairs, in a blinded and independent fashion using CONSORT statement for RCTs. (25) Any discrepancy in the evaluation of the articles was resolved using Delphi methodology, which was coordinated by the principal investigator. Analysis included characteristics of participants (age, country, type of URI), types of intervention (strain of probiotic, CFU dose administrated, time of administration) and reported outcomes (duration of the episodes, number of episodes, number of days with URI, use of antibiotics and scholar absentees)

*Risk of Bias (RoB) in the Included Studies*

Trial quality and RoB in the included studies were assessed according to the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (26), taking into account evidence interpretation using the GRADE approach. This included rating the adequacy of randomization: randomization; allocation concealment; blinding participants; blinding outcomes; incomplete outcome data; selective reporting; vested interest bias and loss of participant follow-up.

*Data synthesis and analysis*

From a statistic point of view, the information was analyzed with the strategy of multiple treatments meta-analysis. We use placebo groups as the central axis for direct comparisons. Indirect comparison was one probiotic against other. Dichotomous outcomes were analyzed with the total number of randomly assigned participants as the denominator. When reported, information on participants that abandoned the studies was included in the analysis. For each potentially eligible study, descriptive statistics of the population characteristics and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

their results were reported, describing the type of comparison as well as the most important clinical and methodological variables.

For each pairwise comparison (direct or indirect), the Hedges standard mean difference (SMD) was calculated for continuous numeric variables, whereas the respective risk ratio (RR) was calculated for dichotomous outcomes. Both were calculated with their respective 95% confidence interval ($CI_{95\%}$). The first meta-analysis were paired comparisons of all published studies, stratified for strain. For this analysis, we used a random effect model, considering that different studies estimated different treatment effects. Concomitantly, we calculated $I^2$ for heterogeneity and its corresponding P value. Thereafter we assembled a NMA, using a random effect model with a Bayesian approach (27,28) and summarized the results using effect sizes and $CI_{95\%}$. We used the adjusted model as described by Salanti et al. (29) Additionally, we calculated the probability of superiority for each "strain of probiotic" intervention through a SUCRA analysis and presented the results in a ranked graph. (30) To estimate the inconsistency (discordance between direct and indirect evidence with a $CI_{95\%}$ that did not include zero), we calculated the difference between the direct and indirect estimates, taking as reference only the constructed indicators that had included a placebo. (31) Finally, we adjusted the model with and without assumptions of consistency and compared the two models in terms of fit and parsimony. (32) In the case of a significant inconsistency we investigated the distribution of clinical and methodological variables that might have been a potential source of heterogeneity or inconsistency in each group of specific comparisons. All analysis and graphic depictions were performed on the version 15.1 of STATA for Mac

**3. Results**

31 RCTs were analyzed (11, 33-62) (Figure 1). 20 studies were considered and 11 were excluded (Tables 1 and 2). Quality of considered studies was moderate to good. (Table 3). A total of 3,635 children were randomized to probiotics (*L. rhamnosus* GG, n=672; *L. rhamnosus* no GG, n=565; *L. casei*, n=562; *L. reuteri*, n=360; *B. lactis*, n= 242; *L. casei* + *S. termophillus* + *L. bulgaricus*, n=314; 12 strains, n=286; L. acidophilus + B. lactis, n=112; *L. acidophilus*, n=110; *L. rhamnosus* + *L. acidophillus*, n=97; *L. fermentum*, n=91; *B. clausii*, n=40; *L. acidophilus* + *B. bifidum*, n=40; and *L. rhamnosus* + *B. lactis*, n=32) and 3,433 to placebo, summarizing a total of 15 nodes of treatment. (Figure 2)

Age of children included in the studies ranged between 7 days to 12 years, with most of children between 1y to 6y. 13 studies were conducted in Europe (including Russia), 4 in Asia, 2 in Latin-America (Chile and Mexico) and one in USA. Duration of interventions was 6 ± 4 months (3 to 24 months).

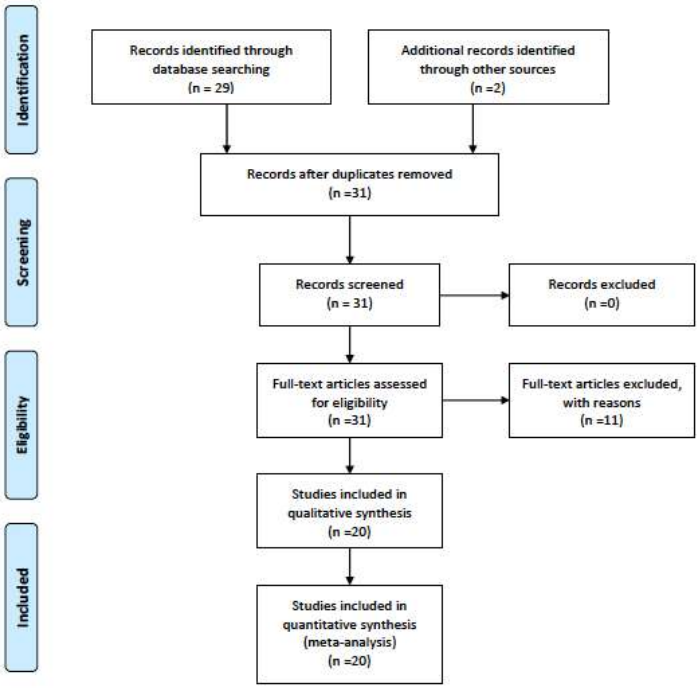
Regarding duration of URI episodes, compared to placebo, the pairwise forest plot showed superiority of *Lactobacillus reuteri* [SMD -0.56 $CI_{95\%}$ (-0.72 to -0.41), p 0.0001] and *Lactobacillus acidophillus* [SMD -0.33 $CI_{95\%}$ (-0.60 to -0.06), p 0.01]; with tendency in case of *L. rhamnosus* GG [SMD -0.14 $CI_{95\%}$ (-0.28 to 0.0), p 0.048] and with no differences for the rest of probiotics. (Figure 3) On the network forest plot (contributive plot analysis) *L.*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

*reuteri* maintains superiority when was compared vs. placebo; showing preventive equivalence when was compared to L. rhamnosus GG, L. casei and BB12 (Figure 4).
In terms of reduction of at least one episode of URIs and reduction of number of days with URIs, comparing with placebo, we observe a significant superiority for L. rhamnosus GG and L. reuteri, with no difference for the rest of probiotics (Figures 5 and 6).
Related to reduction for the use of antibiotics, two single strains [*L. reuteri* RR 0.54, $CI_{95\%}$ (0.32 to 0.93), p 0.02; and L. acidophillus RR 0.30, $CI_{95\%}$ (0.19 to 0.47), p 0.001], and two products with combined strains (*L. acidophilus* + *B. lactis* and *L. rhamnosus* + *B. lactis*) demonstrated superiority vs. placebo (Figure 7). In terms of reduction of absents *L. reuteri* [SMD -2.65 $CI_{95\%}$ (-2.94 to -2.35), p 0.001], *L. rhamnosus GG* [SMD -0.28 $CI_{95\%}$ (-0.48 to -0.08), p 0.007], *Lactobacillus acidophilus* [SMD -1.38 $CI_{95\%}$ (-1.68 to -1.08), p 0.001] and the combination of *L. acidophilus* + *B. lactis* [SMD -1.35 $CI_{95\%}$ (-1.65 to -1.05), p 0.001], showed superiority vs. placebo (Figure 8)
Through funnel plots of the network meta-analysis, we identify a low risk of bias (Figure 9).
Finally, we created hierarchies of effect size on the basis of SUCRA rankings for reduction of duration of URIs, with preventive equivalence for *L. rhamnosus* GG, *L. reuteri* and BB12 (Sucra values of 66.3, 63.0 and 62) (Figure 10).

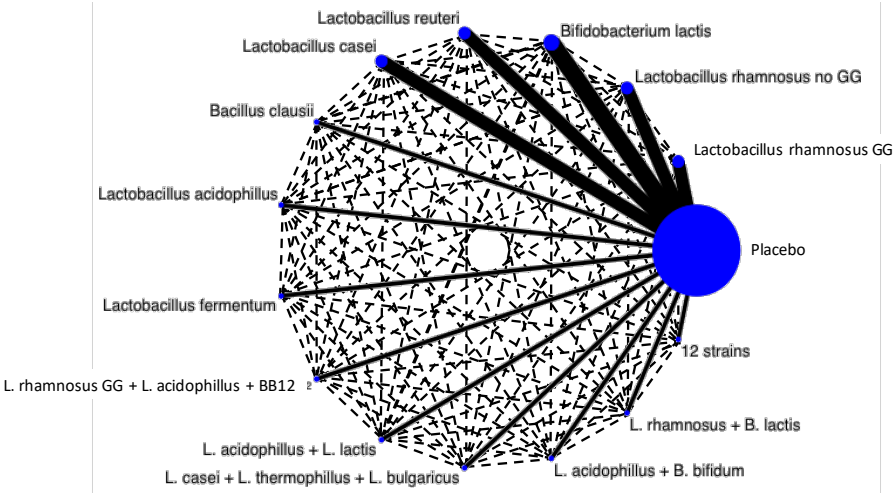
## 3.1. Figures, Tables and Schemes

**Fig. 1 PRISMA Flow Diagram**



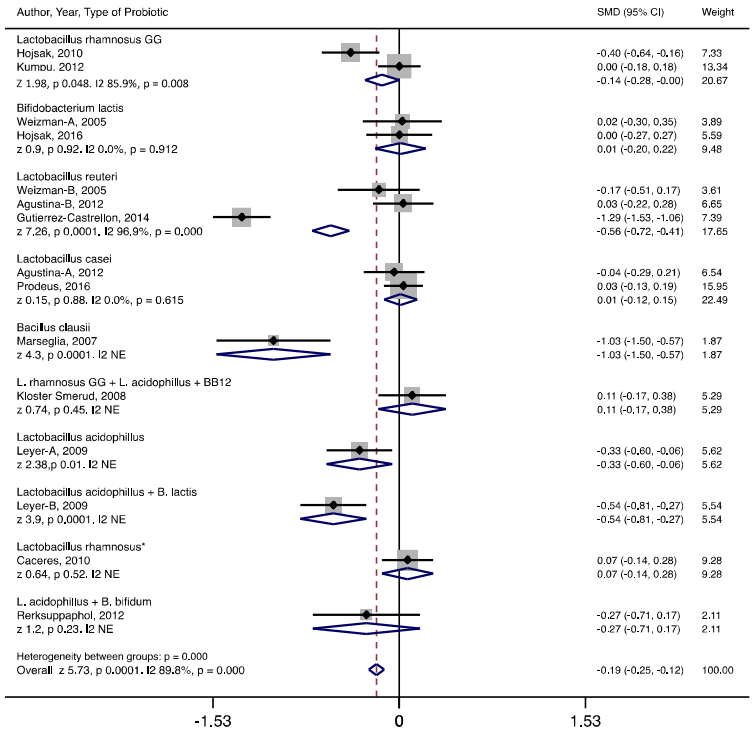
*Preprints* (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

Fig. 2 Network Meta-Analysis on the efficacy of probiotics to reduce the ocurrence of upper respiratory infections in ambulatory children



*Preprints* (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

Fig. 3 Efficacy of probiotics to reduce duration of upper respiratory infections



*Preprints* (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

Fig. 4 Network forrest plot about the efficacy of probiotics to reduce duration of upper respiratory infections

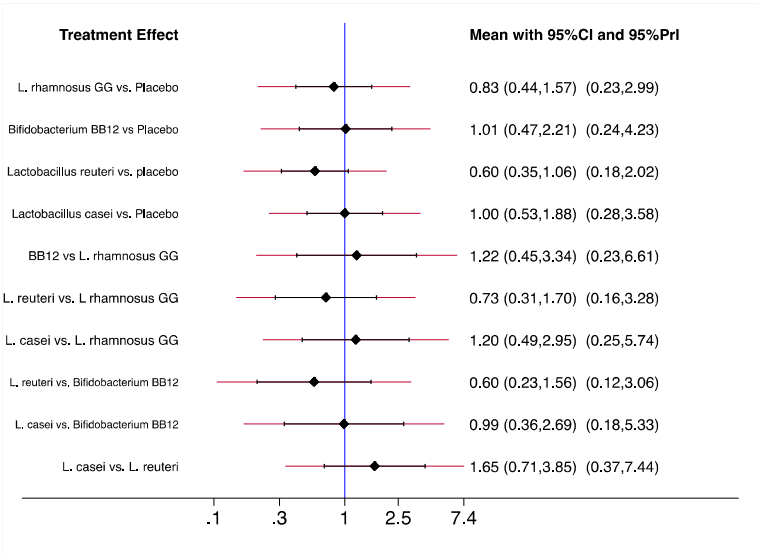
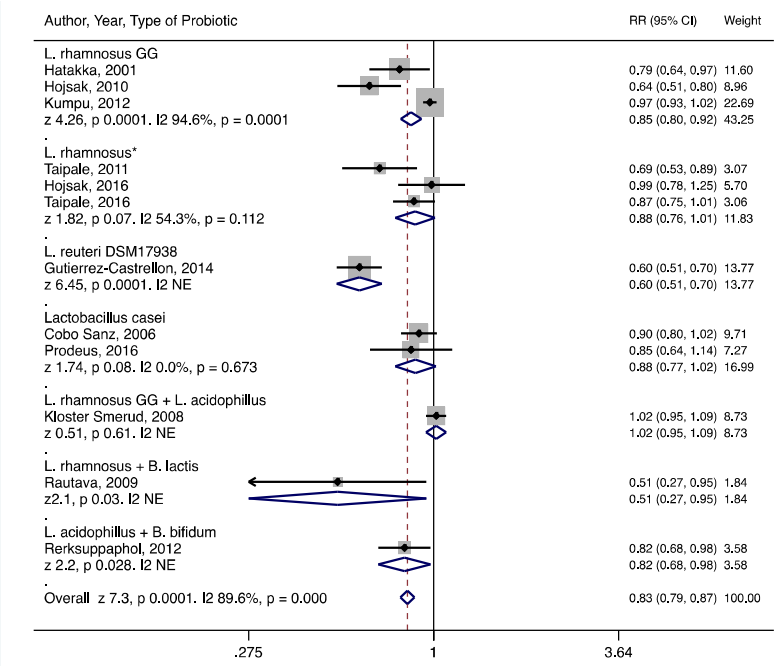



**Fig. 5 Efficacy of probiotics to reduce at least one episode of upper respiratory infections**



*Preprints* (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

**Fig. 6 Efficacy of probiotics to reduce number of days with upper respiratory infections**

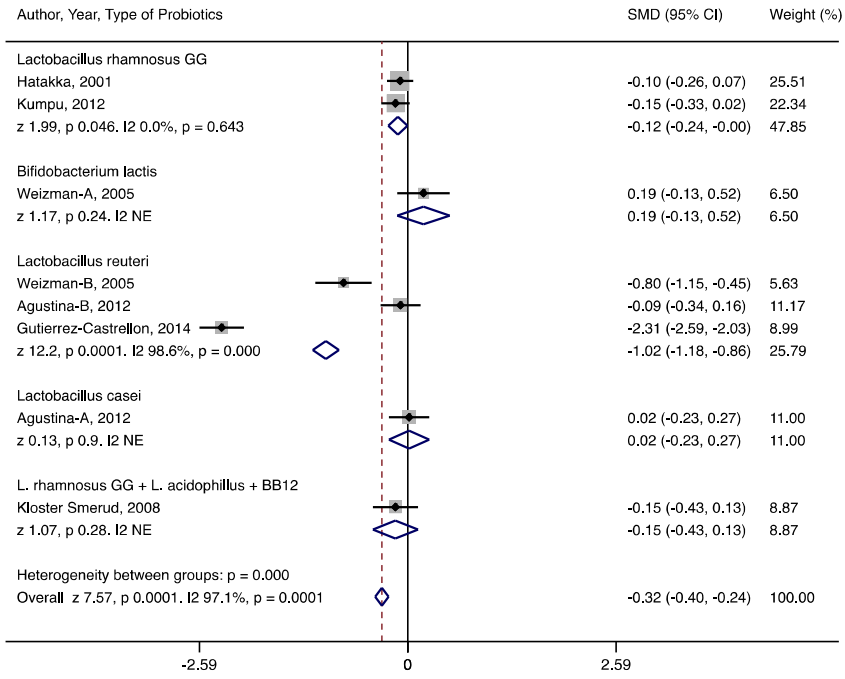
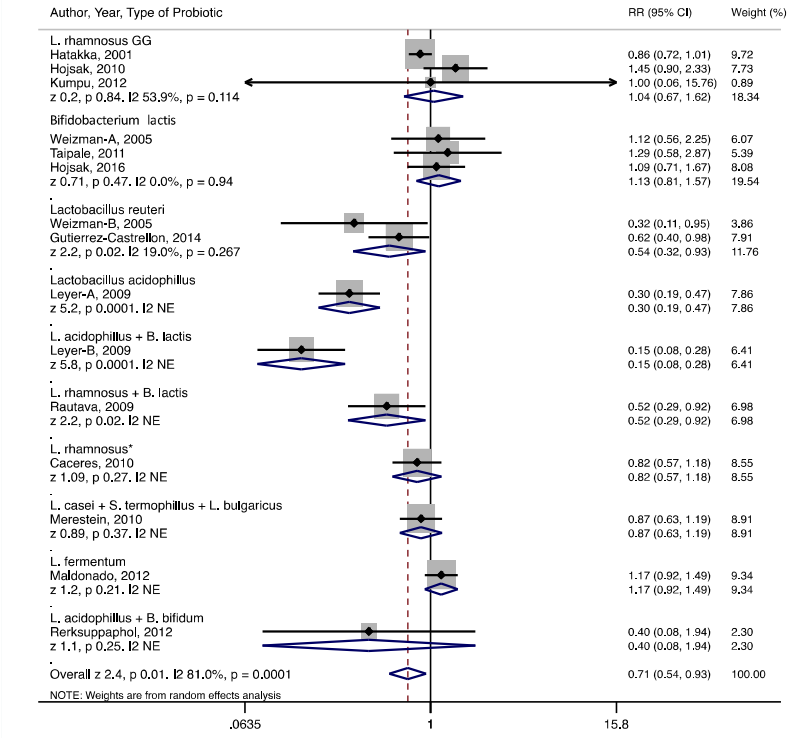
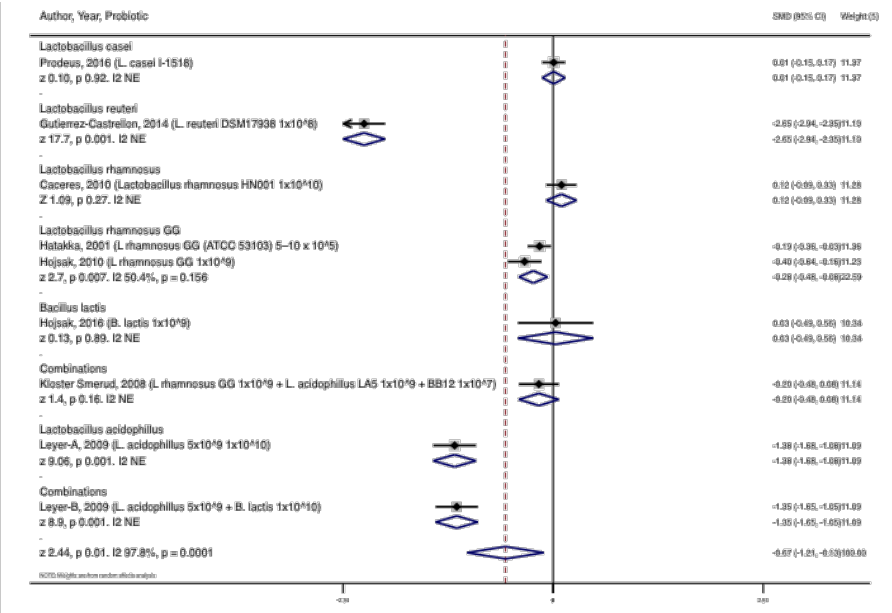



**Fig. 7 Efficacy of probiotics to reduce the use of antibiotics for upper respiratory infections**



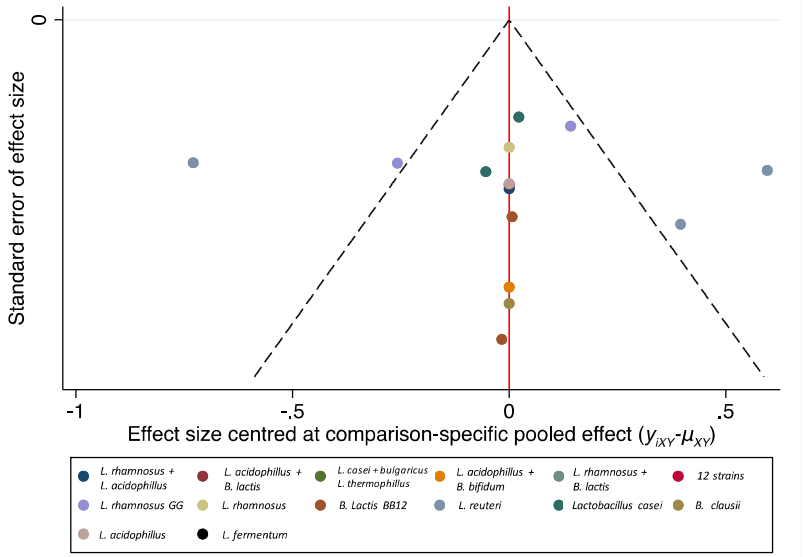
*Preprints* (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

**Fig. 8 Efficacy of probiotics to reduce abseentism secondary to upper respiratory infections**



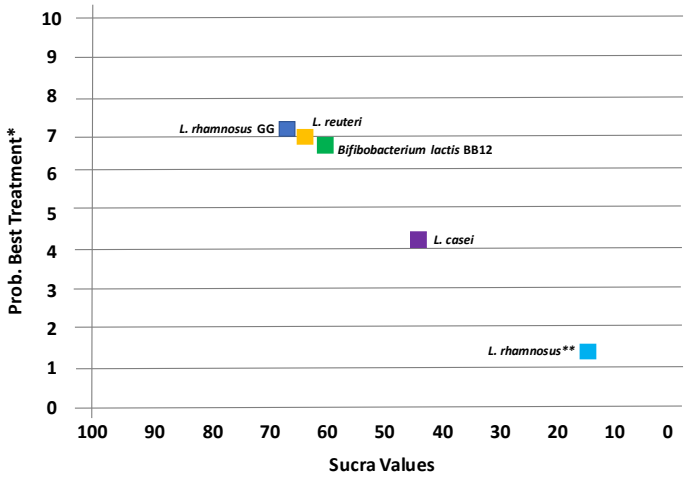
*Preprints* (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

**Fig. 9 Funnel plot about the efficacy of probiotics to reduce duration of upper respiratory infections**



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

**Fig. 10 Best Treatment Analysis for Probiotics to reduce duration of upper respiratory infections**




* Sucra analysis include only probiotic with at least 2 studies on the topic
** Include rhamnosus strain different than GG

**Table 1. Included studies**

| Author | Year | Country | Ages at inclusion | Type of Probiotics | Duration of intervention (months) |
|---|---|---|---|---|---|
| Hatakka, 2001[11] | 2001 | Finland | 1 to 6y | *L rhamnosus GG* (ATCC 53103) 5-10 x $10^5$ | 7 |
| Weizman-A, 2005[33] | 2005 | Israel | 4-10m | BB12 1x$10^7$ | 3 |
| Weizman-B, 2005[33] | 2005 | Israel | 4-10m | *L. reuteri* ATCC55730 1x$10^8$ | 3 |
| Cobo Sanz, 2006[34] | 2006 | Spain | 3 to 12y | *L. casei* DN11401 | 5 |
| Marseglia, 2007[35] | 2007 | Italy | 3-6y | *B. clausii* 2bill spores/5ml | 3 |
| Kloster Smerud, 2008[36] | 2008 | Norway | 1 to 3y | *L rhamnosus* GG 1x$10^9$ + *L. acidophillus* LA5 1x$10^9$ + BB12 1x$10^7$ | 7 |
| Leyer-A, 2009[37] | 2009 | China | 3 to 5y | *L. acidophillus* 5x$10^9$ 1x$10^{10}$ | 6 |
| Leyer-B, 2009[37] | 2009 | China | 3 to 5y | *L. acidophillus* 5x$10^9$ + *B. lactis* 1x$10^{10}$ | 6 |
| Rautava, 2009[38] | 2009 | Finland | 0-2m | *L. rhamnosus* + *B. lactis* 1x$10^{10}$ | 12 |

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 October 2018 doi:10.20944/preprints201810.0002.v1

| | | | | | |
|---|---|---|---|---|---|
| Song Lin-A, 2009[39] | 2009 | Taiwan | 1 to 7y | *L. casei rhamnosus* | 7 |
| Song Lin-B, 2009[39] | 2009 | Taiwan | 1 to 7y | *L. rhamnosus* T-cell1 | 7 |
| Song Lin-C, 2009[39] | 2009 | Taiwan | 1 to 7y | 12 strains* | 7 |
| Caceres, 2010[40] | 2010 | Chile | 1 to 5y | *Lactobacillus rhamnosus* HN001 $1x10^{10}$ | 3 |
| Hojsak, 2010[41] | 2010 | Croatia | 1 to 7y | *L. rhamnosus* GG $1x10^{9}$ | 3 |
| Merestein, 2010[42] | 2010 | USA | 3 to 6y | *L. casei* DN114 $1x10^{8}$ + *S. thermophillus* $1x10^{7}$ + *L. bulgaricus* $1x10^{7}$ | 3 |
| Taipale, 2011[43] | 2011 | Finland | 1-2m | BB12 $1x10^{11}$ | 7 |
| Agustina-A, 2012[44] | 2012 | Indonesia | 1 to 6y | *L. casei* CRL431 | 6 |
| Agustina-B, 2012[44] | 2012 | Indonesia | 1 to 6y | *L. reuteri* DSM17938 $1x10^{8}$ | 6 |
| Kumpu, 2012[45] | 2012 | Finland | 2 to 6y | *L rhamnosus* GG $2x10^{6}$ | 7 |
| Maldonado, 2012[46] | 2012 | Spain | 6m | *L. fermentum* $1x10^{8}$ | 6 |
| Rerksuppaphol, 2012[47] | 2012 | Thailand | 8-13y | *L. acidophillus* + *B. bifidum* $1x10^{9}$ | 3 |
| Gutierrez-Castrellon, 2014[48] | 2014 | Mexico | 6m to 3y | *L. reuteri* DSM17938 $1x10^{8}$ | 3 |
| Hojsak, 2016[49] | 2016 | Croatia | 1 to 8y | *B. lactis* $1x10^{9}$ | 3 |
| Prodeus, 2016[50] | 2016 | Russia | 3-6y | *L. casei* I-1518 | 4 |
| Taipale, 2016[51] | 2016 | Finalnd | 1m | BB12 $1x10^{11}$ | 24 |

| Author | Year | Type of probiotics | Reasons to exclude |
|---|---|---|---|
| Roos K, 2001[52] | 2001 | *alfa streptococci* | Use alfa streptococci |
| Tano K, 2002[53] | 2002 | *alfa hemolytic streptococci* | Use of alfa hemolytic streptococci |
| Hatakka, 2007[54] | 2007 | *Lactobacillus rhamnosus* GG + *Lactobacilus rhamnosus* LC | Treatment of acute otitis media. Not preventive |
| Honeycutt TCB, 2007[55] | 2007 | *L. rhamnosus* GG | Evaluate nosocomial infections in pediatric intensive care patients |
| Cazzola M, 2010[56] | 2010 | *L helveticus* R0052, *B infantis* and *B. bifidum* | Intervention with pre and combination of probiotics |
| Hojsak, 2010[57] | 2010 | *Lactobacillus rhamnosus* GG | Prevent Nosocomial. Not ambulatory |

| | | | |
|---|---|---|---|
| Lethoranta, 2012[58] | 2012 | *Lactobacillus rhamnosus* GG + *L. rhamnosus* LC705þ b-99þP. | Evaluate only Human bocavirus in nasophrainx |
| Di Pierro, 2012[59] | 2012 | *Streptococcus salivarius* K12 | Use of Streptococcus salivarius K12 |
| Cohen R, 2013[60] | 2013 | *Streptococcus thermophilus* + *Streptococcus salivarius* DSM 13084 + *L rhamnosus* LPR CGMCC | Intervention with pre and a combination of probiotics |
| Tappiovara, 2014[61] | 2014 | *L. rhamnosus* GG | Evaluate presence of L. rhamnosus GG in middle ear |
| Hojsak, 2015[62] | 2015 | *Bifidobcaterium animalis* | Prevent Infections in hospitalized children. Not ambulatory |

Table 3. Quality Analysis of Included studies

| Author | Random Sequence Generation | Allocation Concealment | Blinding of participants and personnel | Blinding of outcome assessment | Incomplete outcome data | Selective reporting | Other bias |
|---|---|---|---|---|---|---|---|
| Hatakka, 2001[11] | + | + | ? | ? | ? | + | + |
| Weizman-A, 2005[33] | + | + | + | + | + | + | + |
| Weizman-B, 2005[33] | + | + | + | + | + | + | + |
| Cobo Sanz, 2006[34] | ? | ? | + | + | + | + | + |
| Marseglia, 2007[35] | + | − | ? | + | + | + | + |
| Kloster Smerud, 2008[36] | ? | ? | ? | ? | ? | + | + |
| Leyer-A, 2009[37] | + | + | + | + | + | + | + |
| Leyer-B, 2009[37] | + | + | + | + | + | + | + |
| Rautava, 2009[38] | + | + | + | + | + | + | + |
| Song Lin-A, 2009[39] | + | ? | + | ? | + | + | + |
| Song Lin-B, 2009[39] | + | ? | + | ? | + | + | + |
| Song Lin-C, 2009[39] | + | ? | + | ? | + | + | + |
| Caceres, 2010[40] | + | + | + | + | ? | − | + |
| Hojsak, 2010[41] | + | + | + | + | + | + | + |
| Merestein, 2010[42] | + | + | + | ? | ? | + | + |
| Taipale, 2011[43] | + | + | + | + | + | + | + |
| Agustina-A, 2012[44] | + | + | ? | + | + | ? | + |
| Agustina-B, 2012[44] | + | + | ? | + | + | ? | + |
| Kumpu, 2012[45] | + | + | + | ? | ? | + | + |
| Maldonado, 2012[46] | + | + | ? | + | + | + | + |
| Rerksuppaphol, 2012[47] | + | + | + | + | + | + | + |
| Gutierrez-Castrellon, 2014[48] | + | + | + | + | + | + | + |
| Hojsak, 2016[49] | + | + | ? | + | + | + | + |
| Prodeus, 2016[50] | + | + | ? | + | + | + | + |
| Taipale, 2016[51] | + | + | + | + | + | + | + |

 doi:10.20944/preprints201810.0002.v1

**4. Discussion**

URIs are one of the most common reasons for children in the USA to seek medical care (63), resulting in billions of dollars in health care expenditures; 30 % of URIs are complicated by acute otitis media and 8 % by rhinosinusitis. Considering that in the most of cases the etiology is viral, there had been a lot of debate regarding the early use of antibiotics. Unnecessary use of antibiotics to treat such conditions poses significant financial burden and can result in untoward side effects as well as risk of antimicrobial resistance. On the other hand, inadequate antibiotic treatment in certain cases may increase the risk of suppurative complications and/or invasive infection in this population. (64) Recently a prospective observational study was conducted in order to compare the treatment outcomes between those with and without antibiotic treatment for the uncomplicated URIs and acute diarrhea in young children. 200 previously healthy children presenting with acute uncomplicated URI (aged 2 to 5 years) and 199 children with acute diarrhea (aged 6 months to 5 years) were included. The decision for antibiotic prescription was based entirely on attending physicians' discretion. Antibiotic prescription rates for URI and diarrhea groups were 30.2% and 13.6%, respectively. Univariate analyses indicated that odds ratios (OR) for treatment failure comparing those with and without antibiotics were 0.5 ($CI_{95\%}$ 0.2 to 1.7) and 1.5 (CI95% 0.6 to 3.7) for URI and diarrhea, respectively. Logistic regression analyses indicated that antibiotic treatment was not significantly associated with better treatment outcomes neither URI nor diarrhea cases. Adjusted OR for antibiotic for requirement of additional treatment were 1.06 ($CI_{95\%}$ 0.14 to 8.15) for URI cases. (65) On the other hand, some papers recently published had pointed out the importance of changes in the nasopharyngeal microbiome as significantly associated with the development of URIs. In 2016, the nasopharyngeal microbiome was analyzed in a cohort of 47 healthy children from USA, aged 49 to 84 months trying to establish a relationship between specific profiles and the developing of URIs. All NP samples were profiled for bacterial microbiota using a phylogenetic microarray, and these data were related to demographic characteristics and upper respiratory health outcomes. Authors found that time to develop URI was positively correlated with NP diversity, and children who experienced more frequent URIs exhibited significantly diminished NP microbiota diversity, $p < 0.05$. (66) Very recently 971 specimens collected monthly and during URI and AOM episodes from 139 infants were analyzed. 16S rRNA V4 gene regions were sequenced to determine the interactions between nasopharyngeal microbial pathogens and commensals during viral upper respiratory tract infection (URI) and acute otitis media (AOM). URI frequencies were positively associated with increasing trend in otopathogen colonization. AOM frequencies were associated with decreasing trend in Micrococcus colonization. During URI and AOM, there were increases in abundance of otopathogen genera and decreases in Pseudomonas, Myroides, Yersinia, and Sphingomonas. Otopathogen abundance was increased during symptomatic viral infection, but not during asymptomatic infection. The risk for AOM complicating URI was reduced by increased abundance of Staphylococcus and Sphingobium. (67) In concordance with this rationale, the results of our systematic review with NMA confirms what other publication had concluded about the safety and efficacy of different probiotics as therapeutic and/or preventive options for URIs in children, adults and elderly population. (11, 33-62) Different to previous papers (13-15) our

systematic review with NMA establish a strain-specificity analysis to clearly demonstrate the concept that in this moment is prevalent around the world in terms of strain-specificity, dose-specificity and frequency-specificity for a specific type of health/disease condition, showing the superiority of some strains of probiotics (*L. reuteri, L. rhamnosus GG and Bifidobacterium BB12*), for reduce the burden of the URIs in children. One of the results identified in our study, that is very important to emphasize, is the observed reduction for the use of antibiotics. Since the emergence of antibiotic resistance and the increasing frequency of antibiotic associated adverse, indiscriminate use of antibiotics has emerged as a relevant problem around the world. The prevalence of gastrointestinal, hepatic, renal and even hematological events observed during and after the use of antibiotics represents a health problem associated with very high costs (10), therefore identifying prevention alternatives that help to reduce the use of antibiotics represents a relevant public health strategy. In terms of mode of action and trying to establish a rationale about observed clinical effects, in contrast to how microbiota protects against intestinal infection, where a diversity of mechanisms have been clearly described for how the microbiota inhibits the establishment of colonization by enteric pathogens and fortifies innate and adaptive defenses against those that are able to invade this site (68,69), the mechanistic basis for how the microbiota protects against infection outside the intestine had been limited explained. Microbiota and different probiotics has been shown to protect against systemic and respiratory infection by *Escherichia coli* (70,71), *influenza virus* (72) *Klebsiella pneumoniae* (73,74) *Listeria monocytogenes* (75), *Staphylococcus aureus* (76), and *Streptococcus pneumoniae* (77-79). Very recent research had demonstrated that microbiota and probiotics protects against infection by a) diverse respiratory pathogens; b) enhances respiratory immunity via granulocyte–macrophage colony-stimulating factor (GM-CSF) signaling; c) IL-17A modulated for probiotics primes GM-CSF during infection downstream of the microbiota; d) regulates alveolar macrophage function via GM-CSF; e) GM-CSF stimulates extracellular signal regulated kinase (ERK) to promote bacterial killing by alveolar macrophages; f) modulate pattern recognition receptor (PPR) ligands; and g) stimulate Nod2 to regulate respiratory immunity. Activation of NOD2 seems to represent one of the most powerful mechanisms to protect against respiratory infections. In this sense, research has identified potent activators of NOD2 (activation of 36 ± 1.9), among which are *L. reuteri, L. crispatus and Clostridium orbiscindens* and weak activators (activation of 2.6 ± 0.76) such as *Lactobacillus johnsonii and Lactobacillus. Rhamnosus*. (80)

**5. Conclusions**

Probiotics are safe alternatives to reduce the frequency and duration of URIs in children. They additionally help to reduce the use of antibiotics and the days for absentees.

In terms of strain-specificity there are preventive equivalence for *L. reuteri, L. rhamnosus* and *Bifidobacterium BB2* for reduce the aforementioned outcomes.

Considering the economic impact and burden disease of antibiotic associated side effects, the associated reduction of antibiotics with the preventive use of probiotics for URI in children must be considered as public health strategy.

**Author Contributions:**

Pedro Gutierrez-Castrellon is the main and corresponding author of the publication. He assembled the systematic review protocol, contribute to the statistical analysis of the information obtained and is responsible for the structuring of the publication.
Zvi Weizman contributes to the assembly of the protocol, participated in the analysis of the quality of the evidence, as well as in the structuring and revision of the publication.
Silvia Cruchet and Ener Cagri Dinleyci participated analyzing the publications identified and in the assembly and review of the publication.
Carlos Jiménez-Gutiérrez assembled the database with the numerical information obtained from the articles included in the analysis and together to PGC carried out the statistical analysis and the study of the different meta-analysis
Gabriel López-Velazquez, second corresponding author, contributed in the assembly of the protocol, the review of the publications identified, in the assembly of the different graphs and in the structuring and revision of the manuscript.

**Funding:** Any author receive and funding for the assembly of this paper

**Acknowledgments:** We thank Cochrane affiliate members at Hospital General Dr. Manuel Gea Gonzalez, Mexico for the review of the statistical analysis

**Conflicts of Interest:** All authors declare they have no potential conflicts of interest to disclose

**References**

1. Mulholland K. Global burden of acute respiratory infections in children: implications for interventions. Pediatr Pulmonol 2003;36:469–474. Epub 2003/11/18.
2. World Health Statistics. 2016. Geneva, Switzerland:World Health Organization.
3. Wald ER, Guerra N, Byers C: Upper respiratory tract infections in young children: duration of and frequency of complications. Pediatrics 1991; 87: 129–33.
4. Chonmaitree T, Revai K, Grady JJ, et al.: Viral upper respiratory tract infection and otitis media complication in young children. Clin Infect Dis 2008; 46: 815–23
5. Holstiege J, Garbe E: Systemic antibiotic use among children and adolescents in Germany: a population-based study. Eur J Pediatr 2013; 172: 787–95
6. Tambe MP, Shivaram C, Chandrashekhar Y. Acute respiratory infection in children: a survey in the rural community. Indian J Med Sci. 1999;53:249–53
7. Singh MP, Nayar S. Magnitude of acute respiratory infections in fewer than five children. J Commun Dis. 1996;28:273–8
8. Jain N, Lodha R, Kabra SK. Upper respiratory tract infections. Indian J Pediatr. 2001;68:1135–8
9. Peasah SK, Purakayastha DR, Koul PA, Dawood FS, Saha S, Amarchand R, et al. The cost of acute respiratory infections in Northern India: a multi-site study. BMC Public Health. 2015;15:330

10. Pranita D. Tamma, Edina Avdic, David X. Li, Kathryn Dzintars, Sara E. Cosgrove. Association of Adverse Events With Antibiotic Use in Hospitalized Patients. JAMA Intern Med. 2017;177(9):1308-1315
11. Katja Hatakka, Erkki Savilahti, Antti Pönkä, Jukka H Meurman, Tuija Poussa, Leena Näse, Maija Saxelin, Riitta Korpela. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ 2001;322:1327-32
12. Shan Liu, Pengwei Hu, Xiaoxin DU, Tao Zhou, Xiaofang Pei. Lactobacillus Rhamnosus GG Supplementation For Preventing Respiratory Infections in Children: A Meta-analysis of Randomized, Placebo-controlled Trials. Indian Pediatrics 2013;50:377-381
13. Sarah King, Julie Glanville, Mary Ellen Sanders, Anita Fitzgerald, Danielle Varley. Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis. British Journal of Nutrition 2014, 112, 41-54
14. Hao Q, Dong BR, Wu T. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database of Systematic Reviews 2015, Issue 2. Art. No.: CD006895. DOI: 10.1002/14651858.CD006895.pub3
15. Yizhong Wang, Xiaolu Li, Ting Ge,Yongmei Xiao, Yang Liao, Yun Cui, Yucai Zhang, Wenzhe Ho, Guangjun Yu, Ting Zhang. Probiotics for prevention and treatment of respiratory tract infections in children A systematic review and meta-analysis of randomized controlled Trials. Medicine 2016;95:e4509
16. Marina Azambuja Amaral, Gabriela Helena Barbosa Ferreira Guedes, Matias Epifanio, Mario Bernardes Wagner. Network Meta-Analysis of Probiotics to Prevent Respiratory Infections in Children and Adolescents. Pediatr Pulmonol. 2017;52:833-843
17. Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, Perez LA, Rojas C, Ovalle O, Garcia-Harker JE, et al. Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. Pediatrics 2012;130:e1113–e1120
18. Hatakka K, Blomgren K, Pohjavuori S, Kaijalainen T, Poussa T, Leinonen M, Korpela R, Pitk€aranta A. Treatment of acute otitis media with probiotics in otitis-prone children-a double-blind,placebo-controlled randomised study. Clin Nutr 2007;26:314–321
19. Di Pierro F, Donato G, Fomia F, Adami T, Careddu D, Cassandro C, Albera R. Preliminary pediatric clinical evaluation of the oral probiotic Streptococcus salivarius K12 in preventing recurrent pharyngitis and/or tonsillitis caused by Streptococcus pyogenes and recurrent acute otitis media. Int J Gen Med 2012;5:991–997
20. Honeycutt TCB, El Khashab M, Wardrop RM, McNeal-Trice K, Honeycutt ALB, Christy CG, Mistry K, Harris BD, Meliones JN, Kocis KC. Probiotic administration and the incidence of nosocomial infection in pediatric intensive care: a randomized placebo-controlled trial. Pediatr Crit Care Med 2007;8: 452–458
21. Tapiovaara L, Lehtoranta L, Swanljung E, M€akivuokko H, Laakso S, Roivainen M, Korpela R, Pitk€aranta A. Lactobacillus rhamnosus GG in the middle ear after randomized, double-blind, placebo-controlled oral administration. Int J Pediatr Otorhinolaryngol 2014;78:1637–1641

22. Lehtoranta L, Soderlund-Venermo M, Nokso-Koivisto J, Toivola H, Blomgren K, Hatakka K, Poussa T, Korpela R, Pitk€aranta A. Human bocavirus in the nasopharynx of otitis-prone children. Int J Pediatr Otorhinolaryngol 2012;76:206–211
23. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009;339:b2535
24. Hutton B, Salanti G, Caldwell DM, et al. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. Ann Intern Med 2015;162:777–84
25. Schulz KF, Altman DG, Moher D & CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomized trials. PLoS Med 2010;7:e1000251
26. Higgins JPT, Green S, Cochrane Collaboration. Cochrane handbook for systematic reviews of interventions. Chichester, England; Hoboken, NJ: Wiley-Blackwell; 2008. p 649
27. Lu G, Ades AE. Combination of direct and indirect evidence in mixed treatment comparisons. Stat Med 2004;23:3105–24
28. Ades AE, Sculpher M, Sutton A, et al. Bayesian methods for evidence synthesis in cost-effectiveness analysis. Pharmacoeconomics 2006;24:1–9
29. Salanti G, Higgins JP, Ades AE, et al. Evaluation of networks of randomized trials. Stat Methods Med Res 2008;17:279–301
30. Salanti G, Ades AE, Ioannidis JP. Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. J Clin Epidemiol 2011;64:163–7
31. Salanti G, Marinho V, Higgins JP. A case study of multiple-treatments meta-analysis demonstrates that covariates should be considered. J Clin Epidemiol 2009;62:857–64
32. Dias S, Welton NJ, Caldwell DM, et al. Checking consistency in mixed treatment comparison meta-analysis. Stat Med 2010;29:932–44
33. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. Pediatrics. 2005;115:5-9
34. Cobo Sanz JM, Mateos JA, Muñoz Conejo A. Effect of Lactobacillus casei on the incidence of infectious conditions in children. Nutr Hosp. 2006;21:547-51
35. Gian Luigi Marseglia, Mariangela Tosca, Ignazio Cirillo, Amelia Licari, Maddalena Leone, Alessia Marseglia, Anna Maria Castellazzi, Giorgio Ciprandi. Efficacy of Bacillus clausii spores in the prevention of recurrent respiratory infections in children: a pilot study. Therapeutics and Clinical Risk Management 2007:3:13–17
36. Hilde Kloster Smerud, Charlotte Ramstad Kleiveland, Anette Roll Mosland, Gisle Grave & Stein-Erik Birkeland Effect of a probiotic milk product on gastrointestinal and respiratory infections in children attending day-care. Microbial Ecology in Health and Disease. 2008;20:8085
37. Leyer GJ, Li S, Mubasher ME, Reifer C, Ouwehand AC. Probiotic effects on cold and influenza-like symptom incidence and duration in children. Pediatrics. 2009;124:e172-9
38. Rautava S, Salminen S, Isolauri E. Specific probiotics in reducing the risk of acute infections in infancy--a randomised, double-blind, placebo-controlled study. Br J Nutr. 2009;101:1722-6

39. Lin JS, Chiu YH, Lin NT, Chu CH, Huang KC, Liao KW, Peng KC. Different effects of probiotic species/strains on infections in preschool children: A double-blind, randomized, controlled study. Vaccine. 2009;27:1073-9
40. Paola Caceres, Soledad Montes, Natalia Vega, Sylvia Cruchet, Oscar Brunser and Martin Gotteland. Effects of Lactobacillus rhamnosus HN001 on acute respiratory infections and intestinal secretory IgA in children. Journal of Pediatric Infectious Diseases 2010;5:353–362
41. Hojsak I, Snovak N, Abdović S, Szajewska H, Misak Z, Kolacek S. Lactobacillus GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double-blind, placebo-controlled trial. Clin Nutr. 2010;29:312-6
42. Merenstein D, Murphy M, Fokar A, Hernandez RK, Park H, Nsouli H, Sanders ME, Davis BA, Niborski V, Tondu F, Shara NM. Use of a fermented dairy probiotic drink containing Lactobacillus casei (DN-114 001) to decrease the rate of illness in kids: the DRINK study. A patient-oriented, double-blind, cluster-randomized, placebo-controlled, clinical trial. Eur J Clin Nutr. 2010;64:669-77
43. Taipale T, Pienihäkkinen K, Isolauri E, Larsen C, Brockmann E, Alanen P, Jokela J, Söderling E. Bifidobacterium animalis subsp. lactis BB-12 in reducing the risk of infections in infancy. Br J Nutr. 2011;105:409-16
44. Agustina R, Kok FJ, van de Rest O, Fahmida U, Firmansyah A, Lukito W, Feskens EJ, van den Heuvel EG, Albers R, Bovee-Oudenhoven IM. Randomized trial of probiotics and calcium on diarrhea and respiratory tract infections in Indonesian children. Pediatrics. 2012;129:e1155-64
45. Kumpu M, Kekkonen RA, Kautiainen H, Järvenpää S, Kristo A, Huovinen P, Pitkäranta A, Korpela R, Hatakka K. Milk containing probiotic Lactobacillus rhamnosus GG and respiratory illness in children: a randomized, double-blind, placebo-controlled trial. Eur J Clin Nutr. 2012 Sep;66(9):1020-3
46. Maldonado J, Cañabate F, Sempere L, Vela F, Sánchez AR, Narbona E, López-Huertas E, Geerlings A, Valero AD, Olivares M, Lara-Villoslada F. Human milk probiotic Lactobacillus fermentum CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. J Pediatr Gastroenterol Nutr. 2012;54:55-61
47. Rerksuppaphol S, Rerksuppaphol L. Randomized controlled trial of probiotics to reduce common cold in schoolchildren. Pediatr Int. 2012;54:682-7
48. Pedro Gutierrez-Castrellon, Gabriel Lopez-Velazquez, Luisa Diaz-Garcia, Carlos Jimenez-Gutierrez, Javier Mancilla-Ramirez, Juliana Estevez-Jimenez, Minerva Parra. Diarrhea in Preschool Children and Lactobacillus reuteri: A Randomized Controlled Trial. Pediatrics 2014;133:e904-9
49. Hojsak I, Tokić Pivac V, Močić Pavić A, Pasini AM, Kolaček S. Bifidobacterium animalis subsp. lactis fails to prevent common infections in hospitalized children: a randomized, double-blind, placebo-controlled study. Am J Clin Nutr. 2015 Mar;101(3):680-4
50. Andrey Prodeus, Violeta Niborski, Juergen Schrezenmeir, Alexander Gorelov, Anna Shcherbina, and Alexander Rumyantsev. Fermented Milk Consumption and Common

Infections in Children Attending Day-Care Centers: A Randomized Trial. JPGN 2016;63: 534–543
51. Taipale TJ, Pienihäkkinen K, Isolauri E, Jokela JT, Söderling EM. Bifidobacterium animalis subsp. lactis BB-12 in reducing the risk of infections in early childhood. Pediatr Res. 2016;79:65-9
52. Roos K, Hakansson EG, Holm S. Effect of recolonisation with “interfering” alpha streptococci on recurrences of acute and secretory otitis media in children: randomised placebo controlled trial. BMJ 2001;322:210–2
53. Taipale TJ, Pienihakkinen K, Isolauri E, et al. Bifidobacterium animalis subsp. lactis BB-12 in reducing the risk of infections in early childhood. Pediatr Res 2016;79:65–9.
54. Hatakka K, Blomgren K, Pohjavuori S, et al. Treatment of acute otitis media with probiotics in otitis-prone children—a double-blind, placebocontrolled randomised study. Clin Nutr 2007;26:314–21.
55. Honeycutt TC, El Khashab M, Wardrop RM 3rd, McNeal-Trice K, Honeycutt AL, Christy CG, Mistry K, Harris BD, Meliones JN, Kocis KC.Probiotic administration and the incidence of nosocomial infection in pediatric intensive care: a randomized placebo-controlled trial. Pediatr Crit Care Med. 2007 Sep;8(5):452-8
56. Cazzola M, Pham-Thi N, Kerihuel JC, et al. Efficacy of a symbiotic supplementation in the prevention of common winter diseases in children: a randomized, double-blind, placebo-controlled pilot study. Ther Adv Respir Dis 2010;4:271–8.
57. Hojsak I, Abdovic S, Szajewska H, et al. Lactobacillus GG in the prevention of nosocomial gastrointestinal and respiratory tract infections. Pediatrics 2010;125:e1171–1177
58. Lehtoranta L, Söderlund-Venermo M, Nokso-Koivisto J, Toivola H, Blomgren K, Hatakka K, Poussa T, Korpela R, Pitkäranta. A Human bocavirus in the nasopharynx of otitis-prone children.. Int J Pediatr Otorhinolaryngol. 2012 Feb;76(2):206-11
59. Di Pierro F, Donato G, Fomia F, Adami T, Careddu D, Cassandro C, Albera R. Preliminary pediatric clinical evaluation of the oral probiotic Streptococcus salivarius K12 in preventing recurrent pharyngitis and/or tonsillitis caused by Streptococcus pyogenes and recurrent acute otitis media. Int J Gen Med. 2012;5:991-7
60. Cohen R, Martin E, de La Rocque F, et al. Probiotics and prebiotics in preventing episodes of acute otitis media in high-risk children: a randomized, double-blind, placebo-controlled study. Pediatr Infect Dis J 2013;32:810–4.
61. Tapiovaara L, Lehtoranta L, Swanljung E, M€akivuokko H, Laakso S, Roivainen M, Korpela R, Pitk€aranta A. Lactobacillus rhamnosus GG in the middle ear after randomized, double-blind, placebo-controlled oral administration. Int J Pediatr Otorhinolaryngol 2014;78:1637–1641
62. Hojsak I, Tokic Pivac V, Mocic Pavic A, et al. Bifidobacterium animalis subsp. lactis fails to prevent common infections in hospitalized children: a randomized, double-blind, placebo-controlled study. Am J Clin Nutr 2015;101:680–4
63. Ball TM, Holberg CJ, Aldous MB, Martinez FD, Wright AL. Influence of attendance at day care on the common cold from birth through 13 years of age. Arch Pediatr Adolesc Med. 2002;156:121–6

64. Wald ER. Acute otitis media and acute bacterial sinusitis. Clin Infect Dis. 2011;52 Suppl 4:S277–83
65. Vandepitte WP, Ponthong R, Srisarang S.Treatment Outcomes of the Uncomplicated Upper Respiratory Tract Infection and Acute Diarrhea in Preschool Children Comparing Those with and without Antibiotic Prescription. J Med Assoc Thai. 2015;98:974-84
66. Clark A. Santee, Nabeetha A. Nagalingam, Ali A. Faruqi, Gregory P. DeMuri, James E. Gern, Ellen R. Wald and Susan V. Lynch. Nasopharyngeal microbiota composition of children is related to the frequency of upper respiratory infection and acute sinusitis. Microbiome (2016) 4:34 DOI 10.1186/s40168-016-0179-9
67. Chonmaitree T, Jennings K, Golovko G, Khanipov K, Pimenova M, Patel JA, et al. Nasopharyngeal microbiota in infants and changes during viral upper respiratory tract infection and acute otitis media. PLoS ONE 2017;12:e0180630
68. McKenney, P. T. & Pamer, E. G. From hype to hope: the gut microbiota in enteric infectious disease. Cell 2015;163:1326–1332
69. Littman, D. R. & Pamer, E. G. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 2011;10:311–323
70. Deshmukh, H. S. et al. The microbiota regulates neutrophil homeostasis and host resistance to Escherichia coli K1 sepsis in neonatal mice. Nat. Med 2014;20:524–530
71. Chen, L. W., Chen, P. H. & Hsu, C. M. Commensal microflora contribute to host defense against Escherichia coli pneumonia through Toll-like receptors. Shock 2011;36, 67–75
72. Ichinohe, T. et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc. Natl. Acad. Sci. USA 2011;108:5354–5359
73. Clarke, T. B. Early innate immunity to bacterial infection in the lung is regulated systemically by the commensal microbiota via nod-like receptor ligands. Infect. Immun 2014;82:4596–4606
74. Fagundes, C. T. et al. Transient TLR activation restores inflammatory response and ability to control pulmonary bacterial infection in germfree mice. J. Immunol. 2012;188:1411–1420
75. Khosravi, A. et al. Gut microbiota promote hematopoiesis to control bacterial infection. Cell Host Microbe 2014;15:374–381
76. Gauguet, S. et al. Intestinal microbiota of mice influences resistance to Staphylococcus aureus pneumonia. Infect. Immun. 2015;83:4003–4014
77. Clarke, T. B. et al. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nat. Med 2010;16, 228–23
78. Schuijt, T. J. et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. Gut 2016;65:575–583
79. Gray, J. et al. Intestinal commensal bacteria mediate lung mucosal immunity and promote resistance of newborn mice to infection. Sci. Transl. Med. 2017;9:doi: https://doi.org/10.1126/scitranslmed.aaf9412
80. Rebecca L. Brown1, Richard P. Sequeira, Thomas B. Clarke. The microbiota protects against respiratory infection via GM-CSF signaling. Nature Communications 2017; 8: DOI: 10.1038/s41467-017-01803-x