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The effects of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* on clinical improvement of common cold in children

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ABSTRACT

Children get an average of six common colds per year and irrational antibiotic has been prescribed to 60% of the cases that potentially cause antibiotic resistency. Studies in humans show that probiotics are effective in reducing the severity of common cold symptoms. Probiotics are attributed to an increase of the innate and acquired immune response against the common colds. The study aimed to investigate whether the consumption of *Lactobacillus acidophilus* R0052 and *L. rhamnosus* R0011 influenced the severity of symptoms of the common cold in children. This was a clinical study using a double blind randomized placebo controlled trial design involving 100 children who developed symptoms of the common cold within 24-48 hours before enrollment. Patients in the Probiotics Group (n = 50) received probiotics capsules containing a combination of *L.acidophilus* and *L. rhamnosus* once perday for 7 days. Patients in the Placebo Group (n = 50) received similarly administered capsules containing saccharum lactis. Subjective daily symptom scores for cough, nasal, pharyngeal and systemic symptoms were used as main outcomes. The results showed that the daily mean symptom score during an episode was not significantly different in the Probiotics Group compared the Placebo Group ($p > 0.05$). There was also no significant relative risk reduction in the number of improvement from severity symptoms score of common cold in both of groups (unadjusted absolute % reduction 0, $p = 1$). Moreover, there was no report of adverse events in the Probiotic and Placebo Groups. In conclusion, *L. acidophilus* R005 and *L. rhamnosus* R0011 in the form and dosage studied do not significantly reduce the severity of symptoms of the common cold in children.

ABSTRAK

Anak-anak menderita flu rata-rata enam kali setiap tahunnya dan sekitar 60% kasus flu pada anak diberi antibiotik yang tidak rasional sehingga berisiko timbulnya resistensi. Beberapa penelitian pada manusia membuktikan probiotik efektif mengurangi keparahan gejala flu. Probiotik diyakini dapat meningkatkan respon kekebalan terhadap flu. Penelitian ini bertujuan untuk mengkaji apakah pemberian *Lactobacillus acidophilus* R0052 dan *L. rhamnosus* R 0011 dapat mengurangi keparahan gejala flu pada anak. Penelitian ini merupakan penelitian klinik menggunakan rancangan uji terkontrol plasebo secara acak dan tersamar ganda yang melibatkan 100 anak yang menderita flu selama 24-28 jam sebelum terlibat penelitian. Penderita pada kelompok probiotik (n = 50) diberi kapsul probiotik yang mengandung kombinasi *L.acidophilus* dan *L. rhamnosus* setiap hari sekali selama 7 hari, sedangkan penderita pada kelompok plasebo (n = 50) diberi kapsul yang berisi laktosa.Skor harian subyektif gejala batuk, hidung tersumbat, gangguan tenggorokan dan gejala sistemik digunakan sebagai luaran utama penelitian. Hasil penelitian menunjukkan skor rerata gejala flu harian selama periode pengamatan pada kelompok probiotik tidak nyata dibandingkan skor rerata pada kelompok plasebo ($p > 0.05$). Pengurangan skor risiko relatif tingkat keparahan gejala flu pada kedua kelompok juga tidak berbeda nyata (*unadjusted absolute % reduction* 0, $p = 1$). Selain itu, tidak dilaporkan adanya efek samping baik pada kelompok probiotik

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dan plasebo. Sebagai kesimpulan, *L. acidophilus* R005 dan *L. rhamnosus* R0011 dengan dosis yang digunakan dalam penelitian ini tidak terbukti dapat mengurangi gejala flu pada anak.

Keywords: common cold-children - probiotics - *Lactobacillus acidophilus* - *L.rhamnosus* - clinical trial

INTRODUCTION

Common cold is a viral infection of the upper respiratory tract characterized by symptoms of cough and cold. The most commonly infecting viruses are rhinovirus, coronavirus, influenza viruses, adenoviruses and parainfluenza viruses. Common cold is a self-limited disease that can heal themselves.[1]

Prevalence of common cold in Indonesia in children is three to six times. Common cold is one of the main causes of patient visit at health facilities in Indonesia, accounting 40%-60% of visit for treatment at health care centers and 15% -30% of visits at the outpatient treatment.[2] Common cold significantly affects the health and comfort of the patients as it requires substantial medical costs and an economic burden.[3]

Irrational prescribing of antibiotics has been reported in 60% of cases of upper respiratory infection.[4] This is an important issue in health, as it can cause resistance to antibiotics. Meanwhile, the symptomatic treatment based on the latest from the Cochrane review is not effective.[5]

There have been many experimental data to support the hypothesis that probiotics may provide a beneficial effect on infectious diseases by immunomodulatory immune system.[6] Probiotics enhance the immune system, both locally and systemically[7,8] which can be potentially relevant with the immunomodulatory effects to overcome the common cold infection. However,the scientific evidence on this subject is still very limited. Therefore, clinical studies to obtain the scientific evidence are needed. This study was conducted to evaluate the effect of probiotics administration (*L.acidophilus* R0052 and *L. rhamnosus* R0011) on the clinical improvement of common cold in children.

MATERIALS AND METHODS

Study design

This was a clinical study using a double-blind randomized placebo controlled trial design to evaluate the effect of *L. acidophilus* R0052 and *L. rhamnosus* R0011 on clinical improvement of common cold in children. Subjects were assigned randomly using a computer program to receive probiotics or placebo. The study protocol has been approved by the Medical and Health Research Ethical Committee of Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Written informed consents were obtained from parents of all subjects.

Subjects

Target population in this study were children aged 2-6 years who suffered from common cold, while the accessible population in this study were children with the common cold who were brought by their parents to Primary Health Care Center (*Pusat Kesehatan Masyarakat = Puskesmas*) and met the inclusion and exclusion criteria.

The inclusion criteria referred to children who suffered from common cold according to American Academy of Pediatrics criteria (2006) which includes runny nose, sneezing, mild fever (<39 ^{o}C), decreases appetite, sore throat, cough, headache and malaise, had good health and no preexisting diseases, especially respiratory

related diseases during physical examination. Children were excluded from the study if they were currently having symptoms of an acute otitis media, symptoms of an acute sinusitis, symptoms of lower respiratory diseases, history of acute or chronic diarrhea and suffered from common cold more than two days.

Sample size

Sample size for each group was calculated with Lemeshow formula. Based on previous studies, it is assumed that the proportion of the treatment group had improved the clinical symptoms by 60% and in the Placebo Group by 27.5%.[9] Therefore, the minimum sample size in order to estimate the clinical improvement of commom cold for each group of treatment was 38.

Probiotics preparation

Probiotics or placebo preparation were provided as dry powders packaged into capsules. The probiotics preparation consisted of a combination of *L.acidophilus* R0052 at concentration of $0.1x10^9$colony-forming units (CFUs) per g and *L. rhamnosus*R0011 at concentration of $1.9x10^9$ CFUs per g. The placebo preparation was composed entirely of saccharum lactis. The preparations were made in Hospital Pharmacy Installation of Dr. Sardjito General Hospital, Yogyakarta. All preparations were distributed and stored refrigerated at the study site until the time of use.

Protocol of study

The study was conducted within the study period of November 2011 to January 2012.Two *Puskesmas* in Bantul District, Yogyakarta Special Region i.e, *Puskesmas* of Jetis 2 and *Puskesmas* of Sewon 2 were used as the study site. Subjects suspected of common cold underwent anamnesis and clinical examination conducted by physicians of the *Puskesmas* and were gathered to be selected. An explanation concerning the background, objectives, benefit of the study was given during the selection. The characteristics of subjects who met the inclusion and exclusion criteria were taken and an informed consent was given to the parents to be signed. Subjects were then randomly allocated into Probiotics Group or Placebo Group and were administered once per day for 7 days.The effects of the Probiotics and Placebo Preparations on clinical improvement of common cold were monitored by research assistants daily during 7 days based on a severity of illness score proposed by Hemilä and Douglas.[10]

Statistical and data analyses

During the study, research assistants monitored and recorded symptoms severity score of the common cold consisting scores of cough, colds, sore throat and systemic conditions. Summary of statistics consisting off requencies and proportions were generated for categorical variables (e.g. sex, family number smoking, contact history of common cold). Continous variables (e.g. severity score of cough, colds, sore throat and systemic conditions) were shown by using mean. Univariate analysis was performed to evaluate the characteristics of the subjects, while bivariate analysis using Chi square (X^2) and Kolmogorov Smirnov tests was used to determine the significant differences in symptoms severity score of the common cold between probiotics and placebo groups. Relative risk reduction (RRR) in symptoms severity score of the common cold in the Probiotics Groups, in comparison to the Placebo Group, were calculated. Any subjects dropping out or being lost in the follow-up were still calculated and analyzed. The analysis was accomplished using the Statistical Package for the Social Science (SPSS) version 15.0 with 95% confidence interval.

RESULTS

A total of 100 children suffering from common cold were involved in this study. Subjects were randomized using a computer program to be grouped in either probiotics or placebo groups, with 50 children included in each group. TABLE 1 presents the characteristics of subjects among the two study groups. The characteristics of subjects in the two groups were balanced as indicated with the absence of significant difference in the characteristics of subjects between the Probiotics and Placebo Groups.The severity score of common cold in the Probiotics Group was not significantly different compared to the Placebo Group. Eight subjects in the Probiotics Group and five subjects in Placebo Group were lostin follow-up at seventh day of observation. However, the number of subjects who dropped out during the study did not affect the minimal sample size needed in the study.

TABLE 1. Basic characteristics of research subjects

Variables	Probiotics n(%)	Placebo n(%)	p
Sex			
• Male	22(44)	31(62)	0.071*
• Female	28(56)	19(38)	
Age (year)			
• 2 – 4	29(58)	24(48)	0.316*
• 4 6	21(42)	26(52)	
Family member smoking			
• Yes	27(54)	30(60)	0.54*
• No	23(46)	20(40)	
Contact history of common cold			
• Yes	35(70)	28(56)	0.15*
• No	15(30)	22(44)	
Duration of illness (day)			
• 1	14(28)	16(32)	0.39*
• 2	36(72)	34(68)	
Nutritional status			
• Overweight	0(0)	0(0)	
• Normal	50(100)	47(94)	0.24**
• Undernourished	0(0)	3(6)	
History of allergy			
• Yes	5(10)	45(90)	0.71**
• No	3(6)	47(94)	
Maternal education			
• Elementary School	8(16)	12(24)	
• Secondary School	6(12)	10(20)	0.33*
• Senior High School	17(34)	16(32)	
• University	9(18)	12(24)	
School			
• Yes	23(46)	27(54)	0.55*
• No	27(54)	23(46)	
Compliance			
• Good	45(90)	47(96)	0.71**
• Bad	5(10)	3(6)	
Additional therapy			
• Yes	41(82)	44(88)	0.40*
• No	9(18)	6(12)	
Mean of initial score			
• Cough	50.88	50.12	0.88
• Runny nose	49.06	51.94	0.45
• Sore throat	46.92	54.08	0.18
• Systemic	50.46	50.54	0.99

**Chi square test; **Fischer exact test*

The severity score of common cold symptoms namely cough, cold, sore throat and systemic conditions from firstday to seventh day in the Probiotics Group was not significantly different compared to the Placebo Group ($p>0.05$), as presented in TABLE 2.

TABLE 2. The effects of probiotics on symptoms severity score of common cold observation for 7 days

Days of observation	The research group of symptoms, mean symptom score		
	Probiotics(n=43)	Placebo(n=47)	p
Day 0			
• Cough	50.88	50.12	0.88
• Runny nose	49.06	51.94	0.45
• Sore throat	46.92	54.08	0.18
• Systemic condition	50.46	50.54	0.99
Day 1			
• Cough	45.26	45.72	0.92
• Runny nose	46.49	44.60	0.67
• Sore throat	47.14	44.00	0.52
• Systemic condition	46.48	44.61	0.70
Day 2			
• Cough	44.47	46.17	0.78
• Runny nose	47.17	43.97	0.51
• Sore throat	44.57	46.35	0.71
• Systemic condition	46.58	44.51	0.68
Day 3			
• Cough	44.35	46.55	0.66
• Runny nose	46.36	44.71	0.73
• Sore throat	45.03	45.93	0.86
• Systemic condition	43.59	47.24	0.46
Day 4			
• Cough	44.67	46.26	0.76
• Runny nose	43.19	47.62	0.40
• Sore throat	42.94	47.84	0.31
• Systemic condition	43.36	47.46	0.31
Day 5			
• Cough	44.80	46.14	0.79
• Runny nose	44.42	46.49	0.69
• Sore throat	40.94	49.67	0.68
• Systemic condition	38.36	42.44	0.29
Day 6			
• Cough	44.50	42.59	0.70
• Runny nose	40.12	46.58	0.20
• Sore throat	40.72	46.03	0.20
• Systemic condition	42.24	44.64	0.47
Day 7			
• Cough	43.11	41.94	0.81
• Runny nose	40.54	44.28	0.45
• Sore throat	41.50	43.41	0.60

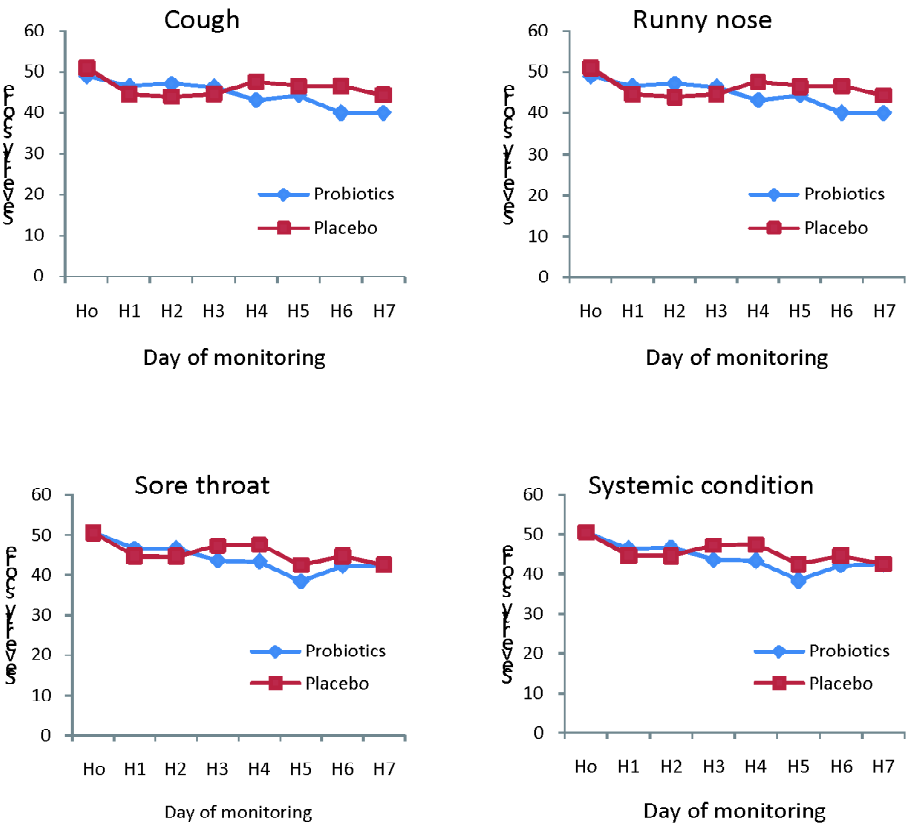

Figure 1. The daily severity score of common cold symptoms in Probiotics and Placebo Groups for 7 days of monitoring

To evaluate the effects of probiotics administration on clinical improvement of common cold, the severity score of the symptoms of both groups at day 7 was determined and compared to base line at day 0 in both groups. On the seventh day of monitoring, the clinical improvement was observed on 11 patients (22%) in the Probiotics Group as well as in Placebo Group (TABLE 3). The total severity score of common cold at the seventh day of monitoring in both groups was similar. The RRR in symptoms severity score of the common cold in the Probiotics Groups, compared to the Placebo Group was 0/78% = 0%. It indicated that the administration of a combination of probiotics consisting of *L. acidophilus* R0052 and *L. rhamnosus* R0011 did not have a significant effect on clinical improvement of common cold in children. Moreover, no side effects due to the probiotics administration was reported during the study.

TABLE 3. Cross table of clinical improvementof common cold symptoms in the Probiotics and the Placebo Groups

Clinical improvement	Groups	
	Probiotic n (%)	Placebo n (%)
Improved	11 (22)	11 (22)
Not improved	39 (78)	39 (78)
	df=1	p=1

DISCUSSION

This study found that the combination of *L. acidophilus* R0052 and *L. rhamnosus* R0011 administration does not affect the clinical improvement of common cold in children.

Probiotics administration in this study was not found to reduce the severity score of cough, cold, sore throat and systemic conditions compared with Placebo group from first day to seventh day monitoring. This result was found to be incontrary to the previous studies.

Probiotics have been investigated widely for health benefits in different disease conditions, especially common colds. de Vrese*et al.*[11,12] reported that the administration of a combination of three strains of probiotics (*L. gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3 dan *B. bifidum* MF 20/5) can reduce duration and severity but not the incidence of common cold episodes. Other study showed that *L. acidophilus* NCFM or a combination *L. acidophilus* NCFM and *B. animalis* can reduce the incidence and the symptoms of common colds in children.[13] It has also been mentioned that the combination of probiotics, vitamins and mineral can reduce duration and severity of common colds through the increase of cellular immune parameters.[14]

Pre clinical and clinical studies have demonstrated that probiotics are effective against viral infection. However, the underlying mechanisms by which probiotics work are not completely understood. The possible antiviral mechanisms of probiotics include the competitive inhibition of virus attachment to the host cell receptor,[15,16] production of metabolites and substances with direct antiviral activities,[17-19] and stimulation of nonspecific and specific immune responses to viral infections.[20-23]

The effectiveness of probiotics can be influenced by various factors such as viability of probiotics and hosts conditions. Probiotics can be effective only if it remains viable until it reaches its destination in the intestine and initiates an immune response. Resistance to gastric acid is an important requirement of probiotics. Gastric acid has a pH of about 2.5, consisting of water (97-99%) mucin (mucus), inorganic salts, and digestive enzymes. The time required to start when bacteria get out of the hull is about 90 minutes. Once the bacteria are successfully through the stomach, they will enter the upper intestinal tract where bile salts are secreted. After traveling through a difficult environment, probiotics bacteria should survive from the bile salts in the duodenum and then colonize in the low intestinal tract.[24] The time required to empty of the small intestine is about 4-5 hours.[25] In addition, the viability of probiotics depends on the storage conditions. Probiotics will be degraded by heat, light, humidity and oxygen during the storage.[26]

The effectiveness of probiotics may also be influenced by various host conditions such as nutritional status, physiological condition, as well as immune system. Nutritional status is associated with a significant impairment of cell-mediated immunity, phagocyte function, immunoglobulin A concentrations and cytokine production that can change the effectiveness of a dietery supplement of probiotics on respiratory tract diseases in children.[27-29] Moreover, the different physiological conditions during lifespan such as gestation, infancy, childhood, adolescence, young adulthood, adulhood and old age influence the immune system that can influence the effectiveness of the probiotics.[30]

Additionally, the effectiveness of probiotics in the treatment and prevention of viral infections is influenced by the dose and duration of administrations and the strain to strain variations. A commonly held assumption is that higher doses of probiotics given for short courses are more effective than lower doses at ameliorating viral infections. However, the dose effects of probiotics on ameliorating the viral infections remain controversial.[30-32] A review proved that probiotics have a positive benefit against viral infections, althoug the strain variation may be relatively large concerning strain properties and efficacy.[33]

CONCLUSION

In conclusion, the administration of a probiotics combination of *L. acidophilus* R0052 at concentration of $0.1x10^9$ CFU sper g and *L. rhamnosus* R0011 at concentration of $1.9x10^9$ CFUs per g once daily for 7 days does not improve the clinical outcome of common cold in children aged two to six years. The severity score of common cold symptoms of children who were given the probiotics is not significantly different compared to the placebo group.

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