



EFFECTS OF HEAT-KILLED PROBIOTIC COMPLEX ON ACUTE DIARRHOEA IN CHILDREN

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ABSTRACT

Probiotics are known in paediatrics as a new alternative treatment for diarrhoea. Some studies have found that a heat-killed probiotic complex (HKPC) has the same potential effects as active probiotics on diarrhoea in children. However, there is little research on this subject. We conducted a study to examine the HKPC effect on duration of illness and degree of recovery from acute diarrhoea in 6–24-month-old children. This study is a randomised, double-blind, controlled clinical trial with children aged 6–24 months with acute diarrhoea. The children were divided into two groups: one received HKPC and the other placebo. Stool samples were collected prior to treatment to determine the presence of rotavirus and the evidence of fat and/or lactose malabsorption. All the children were observed regarding the duration of diarrhoea, stool frequency, and stool consistency until they recovered. A total of 98 children met the selection criteria. Rotavirus was found in more than half of the stool samples (53%). Fat malabsorption was present in 46% of the samples, yet lactose malabsorption was detected in only 8% of the samples. The HKPC group had a shorter recovery time (3 days) than the placebo group (4 days), which is not statistically different ($p=0.100$). There were no significant differences in recovery levels from the first day until the last day of observation ($p=0.487$). According to the result, administration of HKPC has no significant effect on duration of illness and level of recovery in 6–24-month-old children with acute diarrhoea.

1. Introduction

Diarrhoea is the main cause of child morbidity in developing countries. The mortality rate of diarrhoea in children is 1.4 million per year globally, with the highest prevalence in developing countries such as India, Nigeria, Congo, Pakistan, and China (Black *et al.*, 2010). The incidence rate of diarrhoea is high in children under the age of 2, with the peak rate in children aged 6–24 months who start to eat other foods and reduce breast milk intake (Soeparto *et al.*, 1999; Ansari *et al.*, 2012). The most common causes of diarrhoea associated with high mortality are rotavirus, followed by *Cryptosporidium* spp and *Shigella*

spp (Troeger *et al.*, 2017). Cohort studies have shown that nearly all children suffer at least one rotavirus infection before the age of 5, independent of their socioeconomic status (WHO, 2009). Acute diarrhoea caused by infection can reduce lactose absorption. It damages the small intestinal mucosa and decreases the lactase enzyme, which leads to decreased lactose absorption. This, along with decreased fat absorption, leads to increased lactose and fat in the stool (Khani *et al.*, 2012).

Probiotics are known in paediatrics as a new alternative treatment for diarrhoea, due to their beneficial functions in the gastrointestinal tract.

They produce bacteriocin, which acts as a competitive inhibitor, decreasing the bacterial growth and strengthening the tight junctions in the brush border. Some studies have shown that probiotics could favour the recovery phase in virus-associated diarrhoea by enhancing pathogen-specific secretory IgA production and inhibiting viral multiplication (Ohland and MacNaughton, 2010).

A heat-killed probiotic complex (HKPC) is a preparation with inactive probiotic bacteria or killed probiotics. It is more stable in heat and prolonged storage. Though it is inactive, the complex still contains bacteriocin, lactase, and the deoxyribonucleic acid (DNA) of the probiotic bacteria. These have the function of decreasing inflammation and stopping cell necrosis. It has been stated that HKPCs have the same beneficial potential as living probiotics (Ng *et al.*, 2009). A study by Supriatmo (2006) demonstrated that HKPCs are better than living probiotics at shortening the duration of diarrhoea in children.

In this study, we examined the effect of HKPC on the duration of illness and the degree of recovery from acute diarrhoea in 6–24-month-old children.

2. Materials and methods

2.1. Materials

2.1.1. Samples

All 6–24-month-old children with acute diarrhoea (described as watery diarrhoea occurring more than 3 times per day, for less than 3 days before hospitalisation) in the Child Health Department of Dr Soetomo Hospital from April 2008 to August 2008 were included in this study. Children with a history of probiotic, antibiotic, or zinc administration prior to hospitalisation and with severe comorbidities or malnutrition were excluded from this study. The study design described in figure 1.

The duration of illness was determined by the mean duration of diarrhoea in days after being hospitalised until recovery. Recovery level was determined based on frequency of defecation and consistency of stool, and was classified as the following:

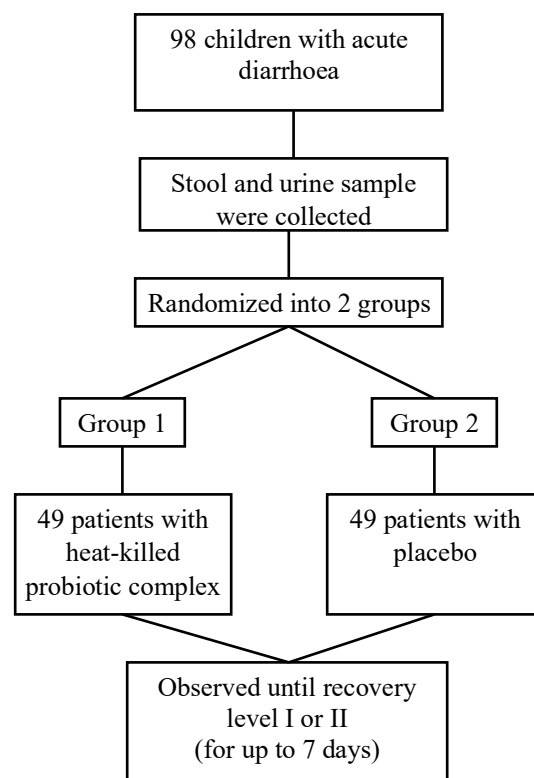


Figure 1. Subject treatment

(1) recovery level I: solid stool consistency no more than 3 times per day; (2) recovery level II: mushy stool consistency no more than 3 times per day; (3) recovery level III: watery stools with lumps no more than 3 times per day; (4) no recovery: watery diarrhoea more than 3 times per day until the seventh day of diarrhoea with standardised treatment. Stool consistency was observed according to the Bristol stool chart.

2.1.2. Heat-Killed Probiotic Complex

In this study, we used a manufactured heat-killed probiotic complex (Dialac®) with tyndallized lyophilisate of *Lactobacillus acidophilus* as an inactive probiotic at a dose of 3×10^{10} CFU/day. This HKPC also contains bacteriocin, lactase enzyme, bacterial CpG-DNA, vitamins B2, B3, B6 and C, thiamine, zinc, calcium, sweetener and fruit flavouring. The product is packaged in sachets with a net weight of 1 gram. The control group received a similar sachet, but it contained only 300 mg of *Saccharum lactis* (placebo).

2.2. Methods

The Ethical Research Commission of Dr Soetomo General Hospital in Surabaya approved this study. This study is a randomised, double-blind, controlled clinical trial in which randomisation was done by a third party. Each child meeting the selection criteria was examined and a stool sample was collected for laboratory analysis. The children were then randomised into two groups: an HKPC group and a control group. They received standard treatment for diarrhoea and were given a sachet of HKPC or placebo twice a day until they reached recovery level I or II. All the patients were evaluated every day in terms of defecation frequency and faecal consistency for up to 7 days until they reached recovery level I or II.

The stool and urine examination was done only once before the HKPC or placebo administration. The urine samples were collected using the sterile urine collector for at least 5 ml. The stool samples were collected using a sterile pot. Approximately 5 grams of stool sample, taken from the middle part of the stool, was sent inside the sterile pot to the laboratory for the examinations within 3 hours. A rotavirus kit (Meridian Bioscience, ImmunoCard STAT!® Rotavirus Devices 750030, USA) was used to detect rotavirus antigen in the stool using the qualitative immunochromatographic assay method. Approximately 0.25 µL (if liquid or semi-solid) or 2 mm³ (if solid) of the stool sample was diluted with 350 µL of sample diluent (a buffer containing 0.1% sodium azide) in a 12x75 mm glass test-tube and homogenised with vortex mixer (QL System, MX-2500 Vortex Mixer, UK) for ten seconds. The diluted sample (as much as 150 µL) was transferred to the sample port then incubated for ten minutes at room temperature (25 to 27°C). The result was obtained by visually reading the control and test zones for the presence or absence of a line at the end of the incubation period. The test result was positive if the test and control lines were both visually detectable.

Every stool sample was also analysed using the Clinitest® tablet (Bayer HealthCare, Mexico)

to detect any lactose malabsorption by detecting unabsorbed sugars in the stool. Approximately 2 grams of the stool was diluted with twice its volume of distilled water (RPI, Distilled Water W20525, USA) in a 12x100 mm glass test-tube, then mixed with a vortex mixer. Fifteen drops of this mixture were transferred to a test-tube, a Clinitest tablet was added, then boiled and wait for 15 seconds after boiling. The result was observed at the end of 15 seconds and compared to the Clinitest® colour chart. The amount of reducing substance present was rated as 0%, 0.25%, 0.5%, 0.75%, 1%, or 2%. A result of 0.5% or more indicated the presence of an abnormal amount of sugar in the stool.

For every stool sample, the floating test was done to detect any fat malabsorption. 3 grams of the stool sample was gently placed in a 100 mL glass flask containing 30 mL of distilled water (RPI, Distilled Water W20525, USA) at room temperature (25 to 27°C), and its floating or sinking position was observed within 30 seconds. If the stool floated, it indicated the presence of steatorrhoea.

Both the stool and urine samples were examined for bacterial identification. The urine and stool samples were cultured in MacConkey agar (Merck Millipore, MacCONKEY Agar 105465, Germany) for 18-24 hours to isolate the bacteria colony. Gram staining was done to confirm and choose the right panel for bacteria identification. The colony then suspended into the Phoenix™ ID broth (BD Diagnostic, Phoenix™ ID broth 246001, USA) with inoculum density 0,5 McFarland (BD Diagnostic, BD PhoenixSpec™ nephelometer 440910, USA). The bacteria identification was using BD Phoenix™ Automated Microbiology System (BD Diagnostic, Phoenix™ 100 instruments 448100, USA) with the panel for gram positive bacteria is PMIC/ID-55 and gram negative bacteria is NMIC/ID-4.

The collected data was analysed statistically using SPSS Windows Release 12.00 software. Descriptive analysis was carried out to compare the duration of illness, frequency of defecation, stool consistency, and recovery levels between the two groups. Lactose and fat malabsorption

tests and the rotavirus test were carried out for the sample median, modus, and proportion. The differences between the two groups were analysed using Chi Square for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables.

3. Results and discussions

A total of 98 eligible patients were included in the study. They were divided randomly into equally sized HKPC and control groups (49 patients each). None of the patients met the exclusion criteria or withdrew from the study. The average age of the selected patients were 11 months old, with almost equal proportions of males (53%) and females (46%). The nutritional status of all the patients in this study was predominantly mild malnutrition (47%). The baseline data is shown in Table 1.

Table 1. Baseline data

	HKPC group (%)	Control group (%)
Age (median)	11	11
Gender		
Male	26 (53%)	27 (55%)
Female	23 (47%)	22 (45%)
Nutritional status		
Overweight	6 (28%)	6 (4%)
Normal	24 (49%)	16 (33%)
Mild malnutrition	19 (39%)	27 (55%)
Dehydration states		
Without dehydration	1 (2%)	1 (2%)
Mild dehydration	9 (18%)	9 (18%)
Moderate dehydration	39 (80%)	39 (80%)
Breast feeding		
Receive breast feeding	28 (57%)	26 (53%)
Not breast feeding	21 (43%)	23 (47%)

The history of diarrhoea prior to hospitalisation is shown at table 2. In this study, most children with diarrhoea were hospitalised after one-day period of diarrhoea with

approximately 6 times diarrhoea per day. More than half of the total children had at least once diarrhoea episode within the last 3 months (73% for both HKPC and control group). The feature of diarrhoea mostly watery with lumpy stool (55% for HKPC group and 65% for control group), none are followed with blood and mucus (78% for HKPC group and 71% for control group), and other symptoms that followed are vomiting, fever and flu-like syndrome.

Table 2. Clinical characteristic

	HKPC group	Control group
Mode duration of diarrhoea (range)	1 (1-3)	1 (0-3)
Mode frequency of diarrhoea	6 (2-11)	6 (1-20)
Diarrhoea consistency		
Entirely liquid	10 (20%)	13 (27%)
Watery with lumpy stool	27 (55%)	32 (65%)
Mushy stool	1 (2%)	0
Diarrhoea characteristic		
Precense of mucus	10 (20%)	13 (27%)
Precense of blood and mucus	1 (2%)	1 (2%)
No blood and mucus	38 (78%)	35 (71%)
Comorbidities		
Vomitting (n)	41	36
Flu-like syndrome (n)	28	36
Fever (n)	28	27
Convulsion (n)	0	0
Episode of diarrhoea in the last 3 months		
One time	36 (73%)	36 (73%)
Two times	7 (14%)	7 (14%)
Three times	2 (5%)	5 (10%)
Four times	4 (8%)	1 (3%)

Data from the stool examination (Table 3) shows that rotavirus was found in more than half of the total samples (53%). This is consistent with the study of Sudarmo *et al.* (2015), which found rotavirus in 80.7% of children aged 6–24 months with diarrhoea. An observation conducted in Jakarta showed that rotavirus prevalence was high among hospitalised children aged 6–23 months with acute diarrhoea,

and it was even more prevalent in the dry season (Soenarto *et al.*, 2009).

Table 3. Stool examinations data

	HKPC group	Control group
Rotavirus test		
Positive	23 (47%)	29 (59%)
Negative	26 (53%)	20 (41%)
Lactose malabsorption		
Positive	46 (94%)	44 (90%)
Negative	3 (6%)	5 (10%)
Fat malabsorption		
Positive	26 (53%)	27 (55%)
Negative	23 (6%)	22 (45%)

Fat malabsorption in acute diarrhoea is more prevalent than lactose malabsorption (46% vs. 8%). Although uncommon in this study, in research done by Nyeko *et al.* (2010), 68% of 3–12-month-old malnourished children with acute diarrhoea displayed lactose malabsorption. This malabsorption is caused by decreased intraluminal digestion due to a reduced absorptive surface area and disturbed enterocyte metabolism. The undigested fat substrate is then hydrolysed by the normal flora in the colon into free fatty acids (Thapar and Sanderson, 2004).

Table 4. Result of the urine and stool culture

	HKPC group (%)	Control group (%)
Urine culture		
No bacterial growth	32 (65%)	27 (55%)
<i>E. coli</i>	13 (27%)	17 (35%)
<i>Proteus</i>	1 (2%)	0
<i>Klebsiella pneumoniae</i>	2 (4%)	2 (4%)
<i>Klebsiella oxytoca</i>	1 (2%)	1 (2%)
Enterobacteriaceae	0	2 (4%)
Stool culture		
No bacterial growth	45 (92%)	46 (94%)
Pathogenic <i>E. coli</i>	0	1 (2%)
<i>Klebsiella pneumoniae</i>	4 (8%)	2 (4%)

The table 4 shows the result of urine and stool culture. From the urine culture, although mostly for both the two groups found no bacterial growth (65% for HKPC group and 55% for control group), but *E. coli* found in 27% HKPC group and 35% in control group. In this study, the stool culture result showed that it is less likely to had bacterial co-infection in children acute diarrhoea (92% for HKPC group and 94% for control group).

3.1. Duration of illness

Between the two groups, the control group needed a longer time to recover (4 days) than the HKPC group (3 days) with range duration for HKPC group is 2-8 days and for control group 2-7 days, but this was not statistically different ($p=0.100$). A similar result was found in another study, which compared the effects of HKPC and active probiotics administration on the duration of illness in children with acute diarrhoea (Indriyani, Juffrie and Setyati, 2016).

Previous studies, in which a higher dosage of heat-killed probiotics than live probiotics was administered to treat acute diarrhoea, found that heat-killed probiotics reduced the duration of acute watery diarrhoea and shortened the length of hospitalisation (Applegate *et al.*, 2013; Supriatmo, 2006). This result showed that HKPCs are as beneficial as live probiotics because they have developed an ability to adhere to the mucus of the human intestine and inhibit the process of diarrheal infection (Supriatmo, 2006).

3.2. Level of Recovery

The recovery level data is shown in Table 5. There were no significant differences in recovery levels between the HKPC and control groups from the first day until the last day of observation ($p=0.487$). This result shows that heat-killed probiotics failed to improve the level of recovery in treating acute diarrhoea in 6–24-month-old children.

Table 5. Recovery level between the HKPC group and control group

	HKPC group	Control group	P value
Recovery level I	12	12	0.487
Recovery level II	31	43	

Many clinical trials on probiotics have also focussed on reduced severity in addition to level of recovery. Severity was represented as stool frequency a few days after administration of probiotics (Applegate *et al.*, 2013). Supriatmo (2006) found that heat-killed probiotics did shorten the duration, but not the stool frequency, of acute diarrhoea in children. Previous studies by Hatta *et al.* (2011) have assessed recovery levels based on the frequency (times/day) and duration of diarrhoea (hours). They found it difficult to assess stool consistency, and this could introduce bias (Hatta *et al.*, 2011). However, in this study, we used the Bristol stool chart to prevent a biased stool consistency assessment.

There are only a few studies of recovery levels based on stool frequency and consistency. Further studies are needed on recovery levels for HKPC administration in acute diarrhoea.

4. Conclusions

Administration of HKPC does not make any significant difference on the duration of illness or recovery levels in 6–24-month-old children with acute diarrhoea, whether or not associated with rotavirus or malabsorption.

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