Indonesian Journal of Clinical Pharmacy, June 2017
Vol. 6 Iss. 2, pg 91–98
ISSN: 2252–6218
Available online at:
http://ijcp.or.id
DOI: 10.15416/ijcp.2017.6.2.91
**Research Article**

# Efficacy of *Bacillus clausii* and *Saccharomyces boulardii* in Treatment of Acute Rotaviral Diarrhea in Pediatric Patients

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## Abstract
Diarrhea disease is considered as major health problem in developing countries. Rotavirus is the most common identifiable viral cause of diarrhea in all children and belongs to Reoviridae family. Rotavirus infection occasionally leads to severe dehydration in infants and children. The objective of the study is to assess the efficacy of *Bacillus clausii* and *Saccharomyces boulardii* on the treatment of rotaviral diarrhea, and also to assess its effect on vomiting and fever in pediatric patients. This study conducted at Rainbow Children's Hospital, Hyderabad, India, from January 2016 until June 2016 and adopts prospective observational parallel study design. From 104 patients enrolled, 80 fulfilled inclusion criteria and 24 were excluded from the study. Patients were divided into two groups based on the treatment. Group I patients were treated with *Bacillus clausii* and Group II patients were treated with *Saccharomyces boulardii*. Total mean duration of diarrhea was significantly shorter in Group II (*S. boulardii*) in comparison with Group I (*B. clausii*). *S. boulardii* significantly ($p \leq 0.005$) decreased the duration of diarrhea which is 25.2 hours over *B. clausii*. Both probiotic preparations were equal in efficacy on treating the vomiting and fever ($p \geq 0.005$). *S. boulardii* and *B. clausii* were well accepted and tolerated by the children and there were no reports of any adverse effects during the study period.

**Keywords:** *Bacillus clausii*, probiotics, rotaviral diarrhea, *Saccharomyces boulardii*

# Efektivitas *Bacillus clausii* dan *Saccharomyces boulardii* dalam Pengobatan Diare Akut akibat Rotavirus pada Pasien Anak

## Abstrak
Penyakit diare merupakan salah satu masalah kesehatan yang paling utama di negara-negara berkembang. Rotavirus merupakan virus famili Reoviridae yang umumnya menjadi penyebab diare pada anak. Infeksi rotavirus dapat menyebabkan dehidrasi berat pada bayi dan anak-anak. Tujuan penelitian ini adalah untuk mengukur efektivitas *Bacillus clausii* dan *Saccharomyces boulardii* pada pengobatan diare rotavirus dan juga untuk menilai pengaruhnya terhadap muntah dan demam pada pasien anak-anak. Studi ini dilakukan di Rumah Sakit Rainbow Children, Hyderabad, India, pada bulan Januari–Juni 2016 dan menggunakan desain penelitian paralel observasional prospektif. Dari 104 pasien yang terdaftar, sebanyak 80 pasien memenuhi kriteria inklusi dan 24 pasien masuk ke dalam kriteria eksklusi. Pasien dibagi ke dalam dua kelompok berdasarkan pengobatan. Kelompok I merupakan pasien yang diberikan *Bacillus clausii* dan Kelompok II merupakan pasien yang diberikan *Saccharomyces boulardii*. Durasi rata-rata diare spada Kelompok II secara signifikan lebih pendek dibandingkan Kelompok I. *S. boulardii* secara signifikan menurunkan durasi diare ($p \leq 0.005$) yaitu 25,2 jam lebih cepat dari *B. clausii*. Kedua preparat probiotik sama efektifnya dalam mengobati muntah dan demam ($p \geq 0.005$). *S. boulardii* dan *B. clausii dapat* diterima dengan baik dan ditoleransi oleh anak-anak, serta tidak ada laporan tentang efek samping selama masa studi.

**Kata kunci:** *Bacillus clausii,* diare rotavirus, probiotik, *Saccharomyces boulardii*

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**Received: 28th September 2016, Accepted: 6th March 2017, Published: 1st June 2017**

## Introduction

Diarrhea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual).[1] Diarrhea disease is considered as major health problem in developing countries.[2] Almost 1.731 billion diarrhea cases occurred in 2010 in children younger than 5 years of age in developing countries, with more than 80% of the diarrhea disease occurring in Africa and Asia. Rotaviral gastroenteritis is associated with a substantial clinical and economic burden in both developed and developing countries. Vaccination is the primary public health intervention for prevention of rotavirus infection.[3] Rotavirus is the most common identifiable viral cause of diarrhea in all children and belongs to Reoviridae family.[4,5] Rotavirus infection occasionally leads to severe dehydration in infants and children.[6] "Probiotic" derived from the Greek, meaning "for life" which evolved to apply to those bacteria that "contribute to intestinal balance". Elie Metchnikoff, known as the "father of probiotics".[7] The probiotic microorganisms should be non-pathogenic in nature resistant to destruction during processing and by gastric acid and bile and able to adhere to intestinal epithelial tissue, colonize in the gastrointestinal tract, produce antimicrobial substances, modulate immune responses and influence human metabolic activities.[8] Probiotics act by five different mechanisms, *i.e.* barrier function, production of antimicrobial substances, competition for adherence, immune modulation, interference with quorum sensing signaling.[9] Preclinical and experimental studies of *S. boulardii* have demonstrated an anti-inflammatory, antimicrobial, enzymatic, metabolic and antitoxic activity.[10]

The objective of this study is to compare the efficacy of *Saccharomyces boulardii* and *Bacillus clausii* in treatment of Rotaviral diarrhea, and also to assess the effect of probiotics on prevention of vomiting and fever. Primary hypothesis of the present study includes *S. boulardii* and *B. clausii* are probotics that have different efficacy in treating Rotaviral diarrhea.

## Methods

The study was approved by the Institutional Ethics Committee - ASNPC/2015-16/03 of the Faculty of Pharmacy, A.S.N Pharmacy College. All participating subjects/guardian signed the informed consent form. This was a prospective observational parallel study design conducted at Rainbow Children's Hospital, Hyderabad, India, from January 2016 to June 2016. The eligible subjects were hospitalized patients over the age from 1 month to 6 years of age who were infected by Rotavirus. We excluded patients with chronic diarrhea (more than two weeks), infections caused by other bacteria, patients treated with antimotility agents for diarrhea, patient who were taken antibiotic/probiotic 7 days prior to hospital admission, ICU admitted patients because we felt that it was difficult to determine the efficacy of probiotics for treatment of diarrhea caused by Rotavirus. We were unable to do a randomized controlled study to compare probiotics with other control group since it would be unethical to provide treatment which is ineffective to patients.

*Saccharomyces boulardii* and *Bacillus clausii* offered to all rotaviral diarrheal patients. Choice of probiotics depends on the responsible physician. Due information was given to all patients before administration of probiotics. On admission, study patient demographic data, type of treatment given, and daily note on frequency of stools, consistency of stools, vomiting, fever and also blood count values were noted and patients were regularly followed up to the hospital discharge. All the information was recorded

according to the information provided by the mother or attendant every morning starting from day 1 to date of discharge. The study subjects were grouped into two groups based on treatment. Group I patients treated with *Bacillus clausii*, Group II patients were treated with *Saccharomyces boulardii*.

The average dose of *Saccharomyces boulardii* was 500 mg/day contain 2.5 million colony forming units (CFU) administered in two divided doses and the average dose of *Bacillus clausii* was 10 ml/day suspension contains 2 billion spores. Both probiotics were diluted in 20 ml of water according to the manufacturers' instructions, and given in two divided doses particularly morning and evening, and minimum duration of administration was 5 days. We used only manufacturer recommended doses in our study. All the patients in study group were treated with third generation of cephalosporin as prophylactic purpose, and received the same diet; those below 6 months of age maintained breast feeding.

The primary outcome measures were the total duration of diarrhea and the number of stools per day and their consistency. Duration of diarrhea shows the time in hours from the first to the last abnormal (loose or liquid) stools preceding a normal stool output. Secondary outcome measures were the incidence and mean duration of vomiting, fever. Data was analyzed using Microsoft Excel to calculate the mean durations. Statistical analysis of the data was performed by using GraphPad stats Software.

## Results

Figure 1 shows the flow of children through the study, a total of 104 children below 6 years of age were admitted during the study period, 80 fulfilled the protocol for the inclusion criteria and divided them into two groups based on treatment (Group I and Group II), each group with 40 members. Table 1 shows demographic data of subjects that 6–12 months age was more susceptible to rotaviral diarrhea without any gender difference.

In our study, on day 1 and 2 stool frequencies were more than 3 times in both groups. On day 3 onwards, the frequency was less than three times a day in 14 (35%) of 40 in the *S. boulardii* group and 1 (2.5%) of 40 in the *B. clausii* group (p=0.001). On day 4, *S. boulardii* was two times more likely to reduce the frequency of stools to less than three per day than *B. clausii* group.

**Table 1 Demographic Characteristics of the Subjects**

| Patient Characteristics | Group I | Group II |
|---|---|---|
| **Age** | | |
| 0–3 months | 00 | 00 |
| 3–6 months | 05 | 01 |
| 6–12 months | 22 | 21 |
| 1–2 years | 09 | 13 |
| 2–3 years | 01 | 03 |
| 3–4 years | 03 | 02 |
| **Sex** | | |
| Male | 20 | 23 |
| Female | 20 | 17 |
| **Average Weight** | 8.8 kg | 9.6 kg |
| **Dehydration Status** | | |
| Mild | 17 | 15 |
| Moderate | 17 | 21 |
| Severe | 06 | 04 |

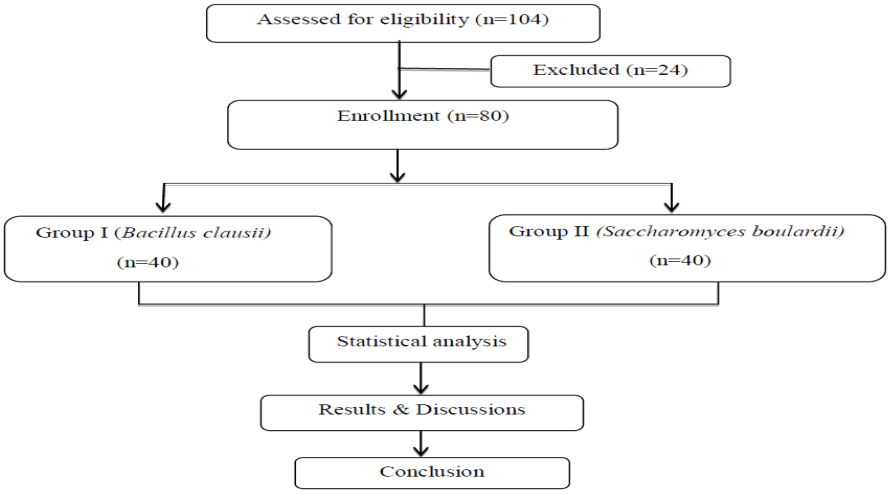


**Figure 1 Study Flow Diagram**

Thirteen patients were treated with other probiotics, four of them were presented with other comorbid conditions (respiratory tract infections, urinary tract infections), three patients had taken antibiotic before hospital admission, two patients were treated with antimotility agents for diarrhea, two patients were having improper medical records.

On day 1 and 2, *S. boulardii* and *B. clausii* had no significant effect on the consistency of stools. However, after day 3, stool consistency was significantly more solid in the *S. boulardii* group compared to *B. clausii*. The daily stool output was slightly decreased in both groups on day 2 (Table 2). On day 3, the mean output of stool was shorter in *S.boulardii* group than *B. clausii* group. Whereas *B. clausii* had reduced the frequency of stool output on day 5. Figure 2 shows there was no significant ($p=0.23$) action on mean duration of diarrhea before and after treatment in Group I where as in Group II patients receiving *S. boulardii*, mean duration of diarrhea before and after treatment was statically significant ($p=0.04$) Table 3 shows the principle outcome of the mean duration of diarrhea was 94.8 hours (2.9 days) in *S. boulardii* group and 69.6 hours (3.95 days) in the *B. clausii* group ($p<0.05$). The duration of diarrhea was significantly altered in Group II by 25.2 hours (*S. boulardii*) when compared to Group I (*B. clausii*) ($p=0.008$).

Our study also confirms both probiotics exhibit equal efficacy in reducing the fever and vomiting (Table 4). No significant difference was observed in these groups. *S. boulardii* and *B. clausii* was well accepted and tolerated by the children and there were no reports of any side effects during the study period.

## Discussion

In an evaluation of two probiotic preparations in children with Rotaviral diarrhea we found substantial differences in efficacy. Total duration of diarrhea in children receiving *S. boulardii* significantly shorter 25.2 hours than *B. clausii*. In our study, we used only single

**Table 2 Primary Outcome Index**

| Groups | Treatment | Days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Group I | *B.clausii* | 10.42 ± 2.89 | 7.5 ± 2.18 | 5.2 ± 2.64 | 2.3 ± 3.02 | 5.25 ± 3.20 |
| Group II | *S. boulardii* | 11.0 ± 5.10 | 6.6 ± 3.14 | 2.9 ± 2.25 | 0.5 ± 1.46 | 0.1 |

Data expressed in mean ± SD

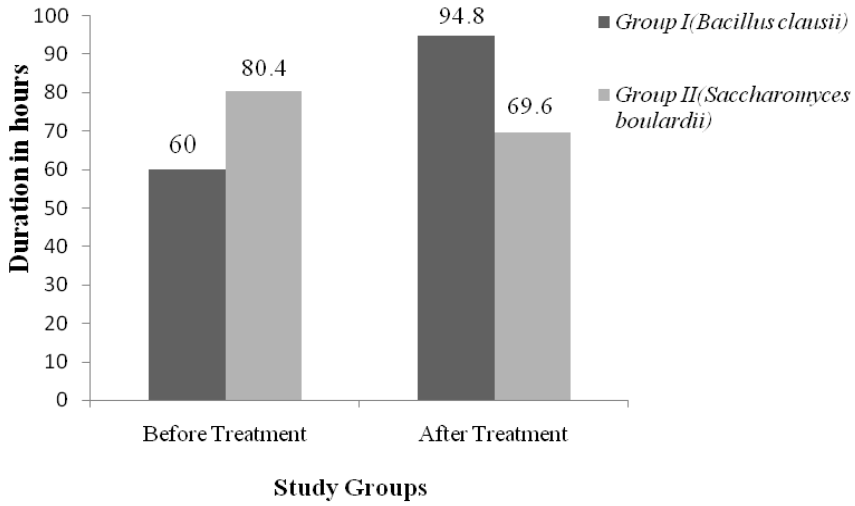


**Figure 2 Comparison of Median Duration of Diarrhea Before and After Treatment**

strain probiotics and first study in comparing single strain probiotics in Rotaviral diarrhea. Limited literature was available for the probiotics efficacy study in Rotaviral diarrhea.

A recent double blind study randomized founds that *S. boulardii* reduces the duration of diarrhea 31.5% patients and probiotics had showed equal efficacy on reducing the fever and vomiting. Randomized Controlled Trails (RCT) conducted in 100 hospitalized children showed that *S. boulardii* treatment for 5 days significantly reduces the mean duration of acute diarrhea and frequency of stools, and normalizes stool consistency.[11] A meta-analysis based on 5 RCTs (619 participants) indicated that *S. boulardii* significantly reduces the duration of acute childhood diarrhea and the risk of prolonged diarrhea compared with control.[12–14]

Acute rotavirus diarrhea remains a major problem in infants and in children resulting in substantial morbidity, mortality, and financial cost. Rotavirus is the agent which most commonly causing diarrhea in children between six months and two years of age, with incidence and severity diminishing after age five.[15,16] Most of the rotavirus-positive children reported were aged between 6–12 months in this study. Rotavirus infection has seasonal characteristics with peak incidence typically in winter.[17] Probiotics are commonly used in viral diarrhea in order to suppress the growth or epithelial invasion of pathogenic bacteria in the human gut, to improve the intestinal barrier function, to modulate the immune system of the intestine, and mediate analgesic functions.[18] Several studies have evaluated on probiotics in the treatment of infectious diarrhea in infants and in children with heterogenous results.[19]

We did not conduct a qualitative and quantitative study of the microbial content of the probiotic preparations in this trial because we wanted to carry out a field trial of clinical

**Table 3 Principle Outcome**

| Group | Treatment | Mean Duration | *p-value |
|---|---|---|---|
| Group I | *B. clausii* | 94.8 ± 1.54 | 0.008 |
| Group II | *S. boulardii* | 69.6 ± 0.69 | |

Duration of diarrhea after treatment, **p-value ≤0.05 when compared to Group I, confidence interval 95%, data expressed in mean ± SD

**Table 4 Mean Duration of Fever and Vomiting After the Treatment**

| Symptoms | Mean Duration (Hours) | | *p-value |
|---|---|---|---|
| | Group I | Group II | |
| Fever | 54.96 ± 1.53 | 45.84 ± 0.81 | 0.0612 |
| Vomiting | 52.8 ± 1.07 | 69.6 ± 0.69 | 0.4155 |

**p-value ≥0.05, confidence interval 95%, data expressed in mean ± SD

effectiveness of commercially available probiotic products that had been prescribed by the paediatrician. *S. boulardii* secretes enzymatic proteins which includes a protease that degrades toxins and a phosphatase that inactivates endotoxins. By reducing chloride secretions it also strengthens tight junctions between enterocytes, promotes maturation of the intestinal brush border membrane and stimulates production of glycoproteins and restores normal levels of short chain fatty acids in the colon which are necessary for absorption of water and electrolytes. *S. boulardii* also decreases inducible Nitric Oxide Synthase (NOS) activity and up-regulates proliferators-activated receptor-gamma, leading to a reduction in intestinal inflammation.[20] Another recent study on *S.boulardii* showed the promising role of probiotic (*S. boulardii*) as an adjuvant to ORS and Zinc in the management of childhood acute diarrhea.[21] Effects of probiotics on vomiting and fever are not clear.[22] Whereas other authors had reported a significant decrease on time of vomiting.[23] The effect of different probiotic species and strains on diarrhea is currently well accepted.[24] However, the dose required to obtain the best results is less clear.

Importantly, *S. boulardii* might interfere with cellular signalling pathways common in many inflammatory conditions and show antimicrobial activity, prevent apoptosis and Tumour Necrotic Factor (TNF) Synthesis, increase immunity.[25] The main limitation of our study was small number of patients and absence of control group.

## Conclusions

Our study demonstrates that *S. boulardii* shorten the duration of diarrhea compared to *B. clausii*, and both probiotics have equal efficacy in treating fever and vomiting in Rotaviral diarrhea, and 6–12 months of age more susceptible to rotavirus. However, more research is needed to extend the clinical application of probiotics and appropriate dose required to obtain best results.

## Acknowledgements

I place my heartfelt gratitude to my respected guide, Prasantha Kumari Mantada, M.Pharm, assistant professor, for her guidance to complete the dissertation work. I sincerely thank Ch.Narasimhulu, M.Pharm and Dr. Prakash, MBBS DNB, for his continuous encouragement and for providing me the necessary facilities and constant support. Finally, I express my heartfelt thanks and gratefulness to all the persons who backed as directly or indirectly throughout of this research work.

## Funding

The study was not funded by any source of grants.

## Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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