

# A Pilot Study On Effect & Role Of Probiotics Supplementation In Dengue Population

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## **Abstract:**

### **Introduction:**

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host while prebiotics are dietary fibre which helps in fermentation and finally results in the proliferation of bacteria. Few studies showed the predominance of one group of bacteria like Bacteroidaceae in dengue with warning signs along with deficiency of Bifidobacterium species, however, the dysbiotic relationship or predominance of either firmicutes or Bacteroides in different categories of dengue is yet to be established. The proposed hypothesis of supplementation of probiotics in dengue to avoid warning signs or complications is secondary to the downregulation of pro-inflammatory cells which can halt the progression of plasma leakage.

**Design:** Prospective, single-centre observational pilot study.

### **Objective:**

To determine whether there is decreased incidence/no incidence of warning signs and complications in diagnosed dengue population following supplementation of probiotics in diagnosed dengue population.

### **Results:**

The correlation between day of the probiotic and the severity of the disease is linear in nature with Pearson's coefficient of correlation (R) 0.544. The linear correlation was statistically significant with P value <0.01.

### **Conclusion:**

Supplementation of Bifidobacterium probiotics in dengue can decrease the incidence of warning signs and severe dengue.

**Keywords:** Dengue, Probiotics, Severity, Dysbiosis.

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## **I. Introduction:**

### **Background:**

Bacteria or bacteriome, fungi or mycome, virus or virome live together in a dynamic equilibrium in the human intestine. The intestinal microbial population helps in nutrient metabolism, stimulates the immune system, and defends the host from pathogens [1]. Gut dysbiosis or alteration of gut microbiota is associated with conditions like inflammatory bowel disease, celiac disease, obesity, metabolic disorder, and the list continues to grow [2]. A healthy microbiota in adult humans is composed of Firmicutes and Bacteroidetes, which together represent approximately 70% of the total microbiota population [3]. Microbiota strongly influences various physiological processes, endocrine and metabolic pathways, expansion and regulation of the immune system, the brain in its cognitive functions, and genome epigenetic changes. Dysbiosis of gut bacteria can lead to various pathologic conditions and to avoid them eubiosis is necessary [4]. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host while prebiotics are dietary fibre which helps in fermentation and finally results in the proliferation of bacteria [5]. Many therapeutic strategies have been and are being used to restore eubiosis which include the administration of prebiotics, probiotics, synbiotics (combination of prebiotics & probiotics) and recent advancements include faecal microbiota transplant (FMT). Dengue infection is a febrile illness caused by one of 4 serotypes of dengue virus and is transmitted by a mosquito named Aedes aegypti or Aedes albopictus. Infection may be asymptomatic or may present with features of a mild febrile illness to a life-threatening shock syndrome. The World Health Organisation revised classification in 2009 classified dengue in to 3 categories which includes dengue without warning signs, dengue with warning signs (abdominal tenderness, hepatomegaly >2 cm (cm), mucosal bleeding and evidence of capillary leak like ascites or pleural effusion) and severe dengue (shock, organ failure and severe bleeding). Numerous viral, host, and vector factors are thought to impact the risk of infection, disease, and disease severity [6]. The duration of impact of probiotics in the transformation of gut microbiota for generating eubiosis is different for various diseases. The earliest time duration reported in different studies was 4 weeks and the average duration was 3 months [7]. Despite a well-known association between gut barrier defect and several diseases, data on the translocation of pathogen molecules, including bacterial DNA lipopolysaccharide (LPS), from the gut to the blood circulation (gut

translocation) in dengue is still less studied. Perhaps, dengue infection might induce gut translocation of several pathogenic molecules that affect the disease severity [8]. Few studies showed the predominance of one group of bacteria like Bacteroidaceae in dengue with warning signs along with deficiency of Bifidobacterium species, however, the dysbiotic relationship or predominance of either firmicutes or Bacteroides in different categories of dengue is yet to be established. The proposed hypothesis of supplementation of probiotics in dengue to avoid warning signs or complications is secondary to the downregulation of pro-inflammatory cells which can halt the progression of plasma leakage [9,10].

**Objective:**

To determine whether there is decreased incidence/no incidence of warning signs and complications in diagnosed dengue population following supplementation of probiotics in diagnosed dengue population.

**II. Methods:**

- **Design:** Prospective, single-centre observational Pilot study

- **Patient population:**

Inclusion criteria:

- All diagnosed dengue patients either by ELISA NS1 or by RT-PCR molecular diagnosis.

Exclusion criteria:

- Pregnancy and Lactation
- Age below 18yrs
- Terminally ill patient
- Any diagnosed comorbidity at the time of diagnosis
- Patients who consumed probiotics/prebiotics/synbiotics within 3 months of diagnosis or consumed after onset of fever before diagnosis of dengue.
- Patients who received antibiotics within 3 months of diagnosis.
- Patients who underwent FMT before diagnosis.
- Known hypersensitivity to probiotics.

- All eligible diagnosed Dengue patients were supplemented with probiotic capsules (Bifidobacterium-based) and were advised to take them twice for 5 days from the day of presentation.
- All the eligible subjects were admitted in the hospital, and categorised during the course of illness into dengue without warning signs, dengue with warning signs and severe dengue as per WHO revised classification 2009.
- Adverse events and complications if any were addressed and managed accordingly.
- Duration:3 months

- **Study variables**

Day of probiotic administration from onset of fever

Features of capillary leak (Ascites, pleural effusion), complications of dengue (as listed in WHO 2009 dengue classification) during the course of illness.

- **Sample size**

50 patients

Based on Sample size  $n = [DEFF * Np(1-p)] / [(d^2 / Z^2_{1-\alpha/2} * (N-1) + p*(1-p)]$

Results from OpenEpi, Version 3, open-source calculator.

- **Statistics:**

Descriptive, correlation and regression (ANOVA) statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean  $\pm$  SD and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance (P value <0.05). Statistics were performed with help of IBM SPSS 20 software.

- Guidelines as per the declaration of Helsinki and good clinical care guidelines were followed during the conduct of study.

### **III. Results:**

Baseline characteristics:

The study included 43 males and 7 females with mean age was 29 years. Among 50 subjects only 4 % received probiotics on day 1, while 36%, 30 %, 22% and 8 % of the patients received probiotics on days 2, 3,4 and 5 respectively (as shown in Figure 1)

39 patients had mild dengue without warning signs, whereas 8 had warning and 3 had severe dengue (as shown in Figure 2). Among 50 patients 4 individuals had gastrointestinal-related (GI) side effects in the form of bloating and dyspepsia, however no individual had a hypersensitivity reaction or constipation.

Association between the day of probiotic and severity of dengue:

The patients who were supplemented with probiotics on the same day of onset of fever were 2 in number and both of them had mild dengue without any warning signs during the course of illness. A total of 18 patients were supplemented with probiotics on day 2, amongst which 17 had mild dengue and only one individual developed warning signs. 15 patients received probiotics on day 3 of the onset of fever, amongst them 14 had no warning signs and only one patient developed warning signs. 11 patients received probiotics on day 4 following symptoms wherein 4 of them had warning signs and 2 patients had severe dengue during their hospitalization. Among 4 patients who received probiotics on day 5, two patients had warning signs and one had severe dengue during their course of hospitalization (as shown in Figure 3).

Correlation between the day of probiotic and severity of dengue:

The correlation between day of probiotic and the severity of disease is linear in nature (as shown in image 4) with Pearson's coefficient of correlation (R) 0.544 (as shown in Table 1). The linear correlation was statistically significant with P value <0.01.

Regression analysis:

Regression analysis performed by ANOVA between day of probiotic and severity of disease showed coefficient of determination (R<sup>2</sup>) of 0.295 which was statistically significant with P value (probability) <0.01. (as shown in Table 2).

### **IV. Discussion:**

The human gastrointestinal tract associated microbes are referred to as the gut microbiome, its role in both health and disease has been the subject of extensive research, establishing its involvement in human metabolism, nutrition, physiology, and immune function. Dysbiosis has been associated with gastrointestinal conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), and wider systemic manifestations of disease such as obesity, type 2 diabetes, and atopy [11]. Despite the proven association of gut translocation of pathogenic molecules into systemic circulation secondary to dengue, the pathogenesis leading to complications/warning signs is much less studied in dengue. A study conducted by Lopez-Santamarina A et al. proved Bifidobacterium species bacteria were deficient in stool samples of individuals who had dengue haemorrhagic fever [12], considering which we tried supplementing Bifidobacterium-based probiotics could potentially reduce complications/warning signs. The mean age of the study was 29 years which is much higher than the published studies of developing countries, the mean age in systemic review of dengue demographics all around the globe corresponds to 22 years [13]. Amongst the diagnosed dengue subjects in this study majority of the population (66%) received probiotics on days 2 and 3 following onset of fever. An observational study conducted in India showed a similar feature where the majority of patients reported to physician on day 3 of illness [14]. The reason for late presentation to physician's clinic might be easy availability of easy availability of antipyretics in India. In this study of 50 admitted diagnosed dengue cases only 8 (16%) had developed warning signs and the incidence of severe dengue was 6% (3 patients). A cross-sectional conducted in India from 2018-2020 had a higher incidence of warning signs, 68% of the total study population had warning signs [15]. A study conducted at the same tertiary centre in 2022 with 150 sample size had a higher incidence of both warning signs (56%) and severe dengue (12%) [16]. The lower incidence of warning signs and severe dengue in our study can be attributed to supplementation of probiotics, to confirm the same correlation and regression analysis were performed. The analysis revealed a linear, statistically significant positive correlation (Confidence Interval of 99%) between day of probiotic supplementation and severity of the disease. Regression analysis performed by ANOVA confirmed the positive correlation is statistically significant and is not by chance. The study also showed that early supplementation of probiotic from the onset of fever (day 1 to 3) had least incidence of warning signs/severe dengue as compared to those who received late (day 4 to 5). We state that supplementation of Bifidobacterium species probiotic might have changed the gut microbiota preventing a higher incidence of warning signs or severe dengue. The fact that this study showed a lower incidence of warning signs/severe dengue in patients who received probiotics early during illness can be attributed to a higher proportion of Bifidobacterium

in gut microbiome among patients who received probiotics early which itself implies for longer duration and thus more concentration in gut microbiota. 4 individuals (8%) of total population had minor GI related side effects which were tolerated well and were treated accordingly. Meta-analysis performed Hempel et al. who reviewed 622 probiotics showed that the risk of side effects of probiotics when compared with placebo is negligible indicating a very good overall safety profile and the meta-analysis also had a higher incidence of GI related side effects (70% of total side effects) in comparison with other systems which were tolerable and easily managed subsequently [17].

#### V. Conclusion:

- Supplementation of Bifidobacterium probiotics in dengue can decrease the incidence of warning signs and severe dengue.
- Supplementation of Bifidobacterium probiotics early during illness is beneficial compared to supplementation late after the onset of symptoms.

#### VI. Limitations:

- Supplementation of Bifidobacterium probiotics as a part of preventive measures in dengue endemic areas to reduce warning signs/ severe dengue requires bigger population.
- Despite the study demonstrating superior effects of supplementation of probiotics, interventional trials and group studies are required to confirm the beneficial effects of Bifidobacterium based probiotics.

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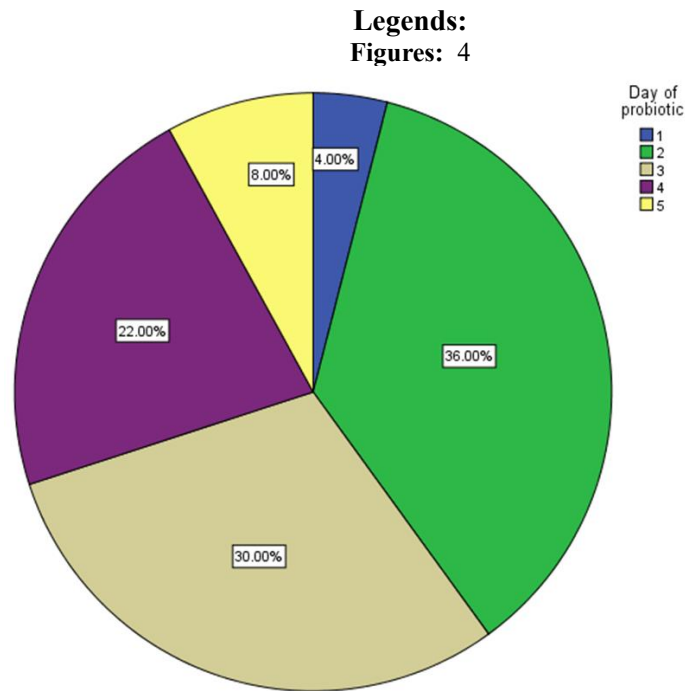


Figure 1 showing piechart of distribution of patients who received probiotics on different days

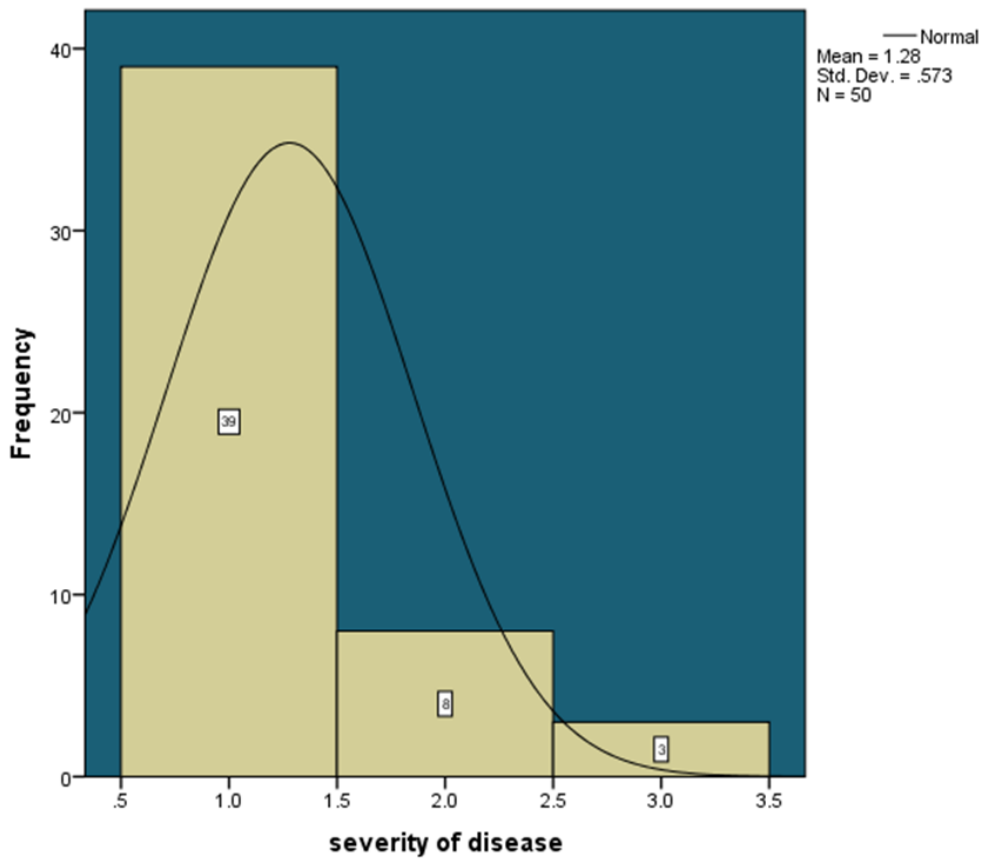


Figure 2 showing histogram of frequency of dengue categories in the study

- 1-Dengue without warning signs
- 2-Dengue with warning signs
- 3-Severe Dengue

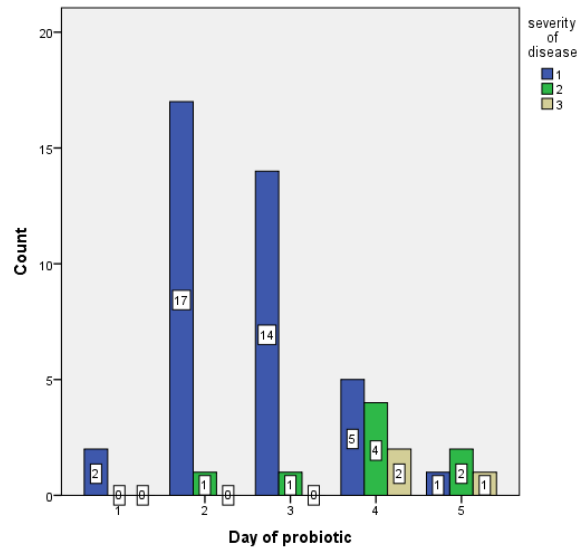


Figure 3 showing histogram of association between day of probiotic and severity of dengue  
 1-Dengue without warning signs  
 2-Dengue with warning signs  
 3-Severe Dengue

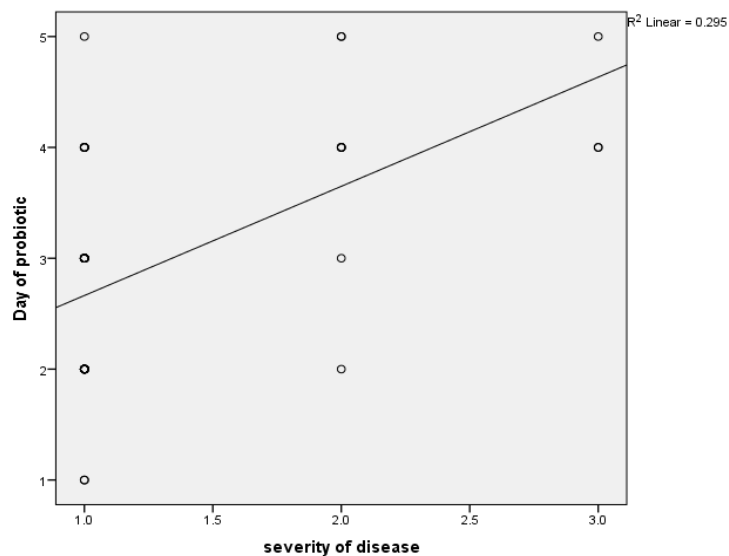


Figure 4 showing linear correlation between day of probiotics and severity of disease

Tables: 2

Table 1 showing Correlation of day of probiotic with severity of disease

| Pearson correlation | Day of probiotic | Grading of severity |
|---------------------|------------------|---------------------|
| Day of probiotic    | 1                | .554**              |
| Grading of severity | .554**           | 1                   |

Correlation is significant at 0.01 level = \*\*

Table 2 showing regression analysis by ANOVA

| Dependant Variable | Independent variable | R value | R <sup>2</sup> | P value |
|--------------------|----------------------|---------|----------------|---------|
| Day of probiotic   | Severity of disease  | 0.554   | 0.29           | <0.01   |

R- Pearson coefficient of correlation  
 R<sup>2</sup>- Coefficient of determination  
 P value – Probability value.