

Effect of Daily *Bacillus subtilis* DE111® Intake on Gastrointestinal Health and Respiratory Infections in Children Attending Day-care: A Randomised, Parallel, Double-blind, Placebo-controlled Study

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ABSTRACT

Children beginning preschool typically have an increased prevalence of gastrointestinal and respiratory infections. This study aimed to evaluate safety and efficacy of the probiotic *Bacillus subtilis* DE111® in gastrointestinal health and respiratory infections in preschool children. In a randomised, parallel, double-blind placebo-controlled study 102 day-care attending children aged 2-6 years received *B. subtilis* DE111® (1 × 10⁹ CFU) or placebo once a day for 8 weeks. Participant diaries were completed by parents and evaluated by investigators to follow the incidence and duration of indicators of gastrointestinal health and respiratory infections as well as any adverse events. Saliva samples were collected at baseline and completion of the intervention to measure sIgA levels. A significant reduction in duration of vomiting (2 days vs. 14 days, p=0.045), duration of hard stools (0 days vs 15 days, p=0.044), and duration of overall gastrointestinal discomfort (18 days vs. 48 days, p=0.0499) was seen. No difference in incidence of respiratory infection was observed (41.3% probiotic vs 36.2% placebo, p=0.60). A statistically significant increase of sIgA levels was observed in the placebo group (1.37-fold, p<0.01), but not in the probiotic group (1.05-fold, p=0.61). Overall, data suggests intake of the probiotic *B. subtilis* DE111® is safe for use in children and supports a healthy gastrointestinal tract with a reduced duration of vomiting, hard stools and overall gastrointestinal discomfort.

Keywords: Probiotics; Children; *Bacillus subtilis*; Gastrointestinal support; Clinical research

INTRODUCTION

The number of children who attend day-care in Europe is approximately 30% for 0-2-year olds and 90% for 3-5-year olds. It has been shown that children attending day-care centres typically have an increased risk of developing gastrointestinal or respiratory infections compared to children cared for at home, particularly during the winter season, often leading to antibiotic use [1-3]. Incidences of such infections warranting a medical visit have been shown to be twice as prevalent compared to the general population of the same age, with the most frequent infections being gastroenteritis (diarrhoea and vomiting) and flu-like illnesses [1]. There is a push globally to minimise the use of antibiotics in the population, therefore ways to support the health of children attending day-care are of great interest.

Defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”, probiotics have the potential to support the maintenance of health in the

population [4]. A number of studies have been carried out in paediatric populations investigating the effects of various strains of probiotics, primarily from genera *Lactobacillus*, *Bifidobacterium* and *Saccharomyces*, on incidence and duration of Gastrointestinal Infections (GII) and upper respiratory infections [5-8]. These studies report mixed results with regards to these types of infections and it is generally acknowledged that the effects of probiotics in the maintenance of health and prevention of infections is strain and dose dependent [9,10].

While the predominant strains of probiotics on the market today are *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* strains, there exists increasing interest and evidence in the use of *Bacillus* species as safe and effective probiotics. As spore-forming bacteria, *Bacillus* confer advantages over other probiotics in that they are able to resist the harsh digestive environment, reaching and colonising the gastrointestinal tract, thus supporting a healthy Gastrointestinal (GI) tract. They are ubiquitous in nature and have been found in the normal microbiota of the gut in healthy adults and children

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[11,12].

Several *Bacillus* species have been reported to show probiotic potential. These include *B. subtilis*, *B. coagulans*, *B. licheniformis* and *B. clausii* [13-15]. *Bacillus subtilis* DE111® is a strain of *Bacillus* probiotic that has been shown to support healthy gastrointestinal function and promote digestive health [15-18]. The aim of this study was to assess safety and efficacy of the probiotic strain *B. subtilis* DE111® in reducing the incidence and/or duration of gastrointestinal and respiratory infections as well as overall GI health in day-care attending children aged 2-6 years old.

MATERIALS AND METHODS

Subjects and study design

This randomised, parallel, double-blind, placebo-controlled study was carried out between April and July 2019 in five paediatric centres in Slovenia. The protocol was approved by the National Medical Ethics Committee (Ministry of Health, Slovenia, 0120-569/2018/4) in Slovenia and registered on clinicaltrials.gov (NCT04077034). Each parent or legal guardian signed an Informed Consent Form prior to enrolment in the study. The study was conducted following the principles of the WMA Declaration of Helsinki (DoH) and ICH-Good Clinical Practice (GCP) guidelines.

Preschool children aged 2-6 years and attending day-care centres were screened by their paediatricians. Children (102) were recruited based on inclusion (generally healthy, attending day-care) and

exclusion (regular medication, immunodeficiency or severe chronic illness, low birth body weight (<2500 g), low gestational age (<37 weeks)) criteria.

Following a 4-week run in period for antibiotic, probiotic and/or immunostimulants wash-out, children were randomly assigned to either the probiotic (N=51) or placebo (N=51) group (Figure 1). A sample of saliva to determine the level of sIgA was taken at baseline and again at the end of the intervention period and analysed using the IgA saliva ELISA test (IBL International GMBH, Germany). Parents of the children recorded the occurrence and duration of symptoms of Respiratory Infections (RI) and GI symptoms through use of a participant diary during an 8-week intervention period and again during a 4-week follow-up period. Any additional Adverse Events (AE) were recorded during the entire 12 weeks of the study to evaluate and confirm the safety of the product. Diaries were assessed by the investigators and reviewed for evaluation of the described symptoms. Product consumption adherence was also recorded in the participant diary. Nine children were excluded due to either study requirements or loss-to-follow-up. The remaining 93 children completed the study and their data were included for statistical analysis (Figure 1) as the Intention to Treat (ITT) population. An additional four children were excluded for not completing the wash-out period for antibiotics and eight children were excluded for long-term use (8-12 weeks) of immunostimulants prior to recruitment. In total, 81 children were included in the Per Protocol (PP) analysis (Figure 1).

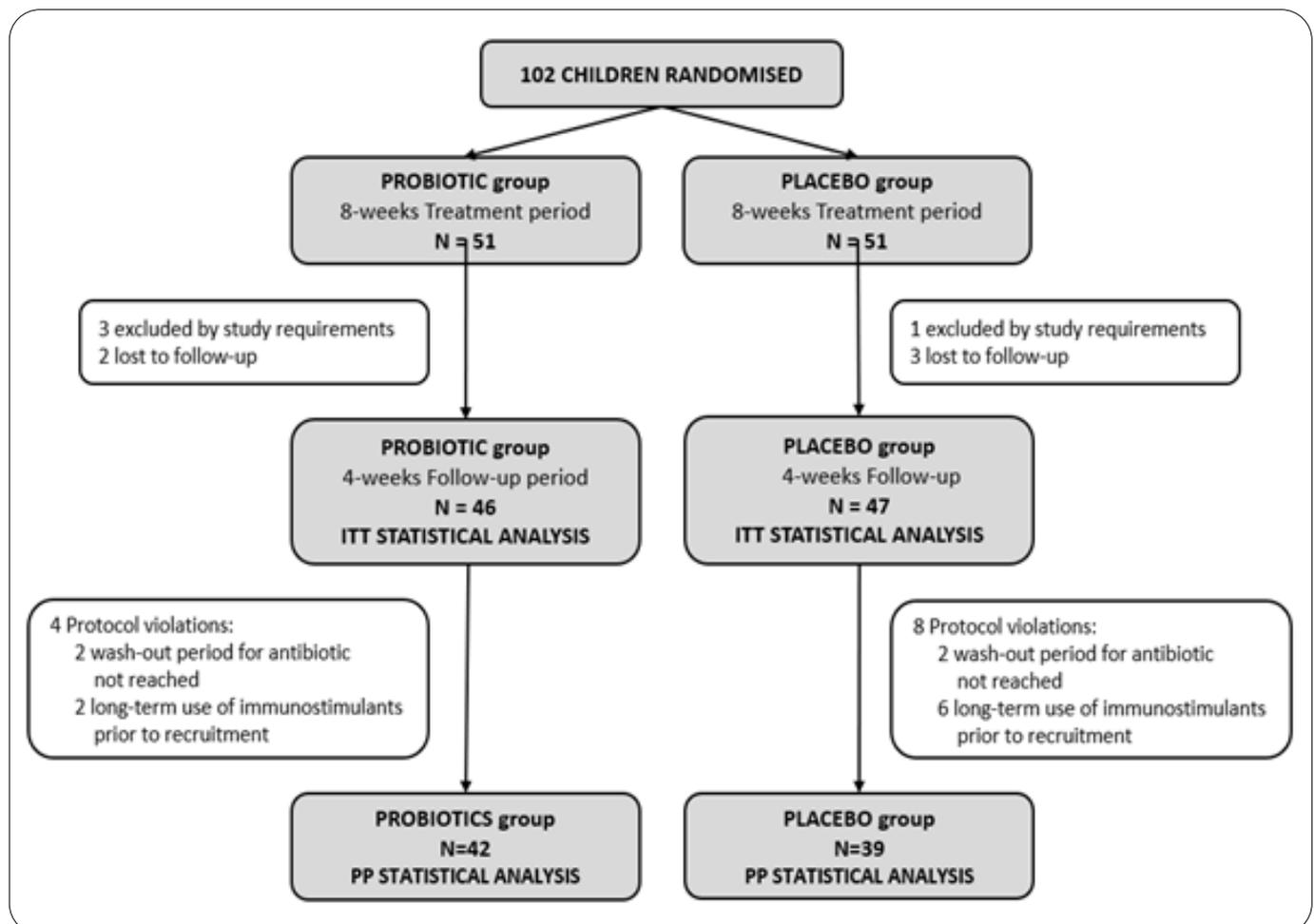


Figure 1: Flowchart of the study profile.

Study product

The intervention consisted of daily consumption of a 1.4 g quick melt stick pack containing either the commercial probiotic strain *B. subtilis* DE111® (1 × 10⁹ CFU/dose) or tapioca maltodextrin (placebo) for 8 weeks.

Safety analysis

Analysis of reported AEs throughout the 12-week study period (8-week intervention and 4-week follow-up), was performed on all 93 participants who completed the study. Adverse events were defined as events not related to, or evaluated in, the objectives of the study under GII, GI health or RI symptoms.

Study outcomes

Outcomes were presented as incidence and duration of GII, RI, individual GI symptoms (diarrhoea, hard stool, constipation, bloating, vomiting) and combined GI symptoms (“gastroenteritis symptoms”: which included diarrhoea and vomiting; and “GI discomfort”, which included diarrhoea, hard stool, constipation and bloating). Gastrointestinal infections were defined as diarrhoea (the occurrence of three loose stools as defined by Bristol stool chart 6 or 7 within 24 hours) lasting ≥ 2 days, occurring with other symptoms of vomiting and/or fever. Respiratory infections were defined as 2 respiratory symptoms lasting ≥ 2 days; 1 symptom lasting ≥ 3 days; 2 symptoms lasting ≥ 1 day accompanied with fever (≥ 37.5°C) or prescription of antibiotics for RI. Levels of salivary IgA (sIgA) from baseline to end of intervention were evaluated.

Statistical analysis

Data were analysed in IBM SPSS Statistics v 26.0 (IBM Corp). Data analysis was carried out both on the ITT and PP populations. Data are presented for two time periods: intervention period (8 weeks) and follow-up period (4 weeks).

The incidence data were reported as frequency and proportion of children with an episode. The difference between placebo and probiotic group for these variables was assessed using Chi-square test with correction for a 2 × 2 contingency table. Additionally, when the sum of occurrences in both study arms was 10 or lower, Fisher's exact test was applied.

Data expressing duration of symptoms (in days) were reported and treated as numerical variables and an assumption of normality distribution was tested. A nonparametric Mann-Whitney's test was used to assess the difference between probiotic and placebo group. Duration was reported as total number of days with infection/symptoms and maximum number of days with infection/symptoms per child for each group.

The measurement data for sIgA concentrations were presented as median for baseline and end of intervention for each group. For the end of intervention, saliva samples collected outside the stipulated collection timeframe (day 54 to day 58) were excluded from the analysis. A nonparametric Mann-Whitney's test was used to assess the difference between probiotic and placebo group.

RESULTS

Baseline characteristics

A total of 102 children were randomised to the placebo or probiotic group with 93 completing the study (ITT population) (Figure 1). There were no statistical differences in sex, age, height, weight, heart rate, respiratory rate, body temperature or in “time of attending day-care” characteristics between groups (Table 1).

Table 1: Baseline characteristics of children.

Characteristics	DE111®	Placebo
	(N=46)	(N=47)
Gender		
Female	22 (47.8%)	28 (59.6%)
Male	24 (52.2%)	19 (40.4%)
Age (months)	54.3 (13.2)	53.7 (13.1)
Height (cm)	108.3 (8.9)	107.3 (8.7)
Weight (kg)	19.5 (3.8)	18.5 (3.6)
Heart rate (beats/min)	97.8 (13.4)	99.2 (12.0)
Respiratory rate (breaths/min)	25.6 (7.1)	26.6 (6.9)
Body temperature (°C)	36.7 (0.3)	36.6 (0.3)
Day care attendance < 24 months	13 (28%)	9 (19%)
Note: Data are presented as mean (± standard deviation) or absolute number (percentage).		

Safety of *B. subtilis* DE111®

In total, AEs were reported for 44 children: 25 children (54.3%) from probiotic and 19 (40.4%) from placebo group. The incidence of individual AEs was up to 6 cases per specific event and did not differ between the groups or from generally occurring health events in preschool children. All AEs were assessed and deemed unrelated to the product or study.

Effects of *B. subtilis* DE111® on GII and GI symptoms

Analysis of the ITT population showed a trend of reduction in both the incidence of GII (8.7% probiotic vs 19.1% placebo, $p=0.23$; Table 2) and the duration of GII (11 days vs 30 days, $p=0.14$, Table 3) of more than 50% in the probiotic group.

The incidence (Table 2) and duration (Table 3) of several individual GI symptoms were also followed during the study. A significant reduction in the duration (2 days probiotic vs 14 days placebo, $p=0.045$) of vomiting and of hard stools (0 days probiotic vs 15 days placebo, $p=0.044$) was observed. The incidence of vomiting (4.3% probiotic vs 17% placebo, $p=0.091$) and hard stool (0 probiotic vs 8.5% placebo, $p=0.12$) showed a trend of reduction, but without statistical significance. Additionally, intake of the probiotic showed a trend of reduction in the incidence and duration of symptoms of gastroenteritis (13% probiotic vs 27.7% placebo, $p=0.12$ and 14 days probiotic vs 36 days placebo, $p=0.082$; respectively), although not statistically significant. Intake of the probiotic also statistically significantly reduced the duration (18 days probiotic vs 48 days placebo, $p=0.0499$) of overall GI discomfort. The incidence GI discomfort showed no statistically significant difference between the probiotic group and the placebo group.

The PP analysis revealed similar results to the ITT population (Tables 2 and 3). A statistically significant lowering of the incidence (4.8% probiotic vs. 20.5% placebo, $p=0.043$) as well as duration (2 days probiotic vs 14 days placebo, $p=0.029$) of vomiting was observed. Probiotic intake showed a trend of lowering the incidence

(7.1% probiotic vs 20.5% placebo, $p=0.11$) and duration (8 days probiotic vs 28 days placebo, $p=0.070$) of GII as well as reducing the duration of symptoms of gastroenteritis (12 days vs. 34 days, $p=0.057$), although the difference was not statistically significant.

Table 2: Incidence of Respiratory Infections (RI) and Gastrointestinal Infections (GII) and symptoms during the 8-week intervention period.

Outcome	ITT population		PP population	
	DE111®	Placebo	DE111®	Placebo
RI	20 (43.5%)	17 (36.2%)	18 (42.9%)	16 (41.0%)
GII	4 (8.7%)	9 (19.1%)	3 (7.1%)	8 (20.5%)
Diarrhoea (Bristol 6-7)	5 (10.9%)	8 (17%)	4 (9.5%)	6 (15.4%)
Hard stool (Bristol 1-2)	0 (0%)	4 (8.5%)	0 (0%)	2 (5.1%)
Constipation	1 (2.2%)	1 (2.1%)	1 (2.4%)	1 (2.6%)
Bloating	2 (4.3%)	3 (6.4%)	2 (4.8%)	3 (7.7%)
Vomiting	2 (4.3%)	8 (17.0%)	2 (4.8%)*	8 (20.5%)
Diarrhoea and vomiting	6 (13.0%)	13 (27.7%)	5 (11.9%)	11 (28.2%)
GI discomfort	7 (15.2%)	15 (31.9%)	6 (14.3%)	11 (28.2%)

Note: ITT population: DE111 N=46, placebo N=47. PP population: DE111 N=42, placebo N=39. Data are presented as absolute number (%). * $p<0.05$.

Table 3: Duration of Respiratory Infections (RI) and Gastrointestinal Infections (GII) and symptoms during the 8-week intervention period.

Outcome	ITT population		PP population	
	DE111®	Placebo	DE111®	Placebo
RI	250 (46)	105 (17)	230 (46)	102 (17)
GII	11 (3)	30 (5)	8 (3)	28 (5)
Diarrhoea (Bristol 6-7)	12 (3)	22 (5)	9 (3)	20 (5)
Hard stool (Bristol 1-2)	0 (0)*	15 (5)	0 (0)	8 (5)
Constipation	2 (2)	1 (1)	2 (2)	1 (1)
Bloating	4 (3)	10 (5)	4 (3)	10 (5)
Vomiting	2 (1)*	14 (5)	2 (1)*	14 (5)
Diarrhoea and vomiting	14 (4)	36 (6)	11 (4)	34 (6)

GI discomfort	18 (6)*	48 (8)	15 (6)	39 (8)
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Note: ITT population: DE111 N=46, placebo N=47. PP population: DE111 N=42, placebo N=39. Data are presented as total number of days (maximal duration). * $p<0.05$.

RI analysis

Analysis of the ITT population revealed no difference in the incidence (43.5% probiotic vs 36.2% placebo, $p=0.61$; Table 2) nor duration (250 days probiotic vs 105 days placebo, $p=0.19$; Table 3) of RI between the groups. The PP analysis showed similar results to the ITT population with no difference in the incidence of RI or the duration of RI between the two groups.

sIgA analysis

No differences were observed in sIgA levels at baseline between the probiotic and placebo groups (22.7 mg/L probiotic vs 24.0 mg/L placebo, $p=0.90$; Figure 2). No change was seen from baseline to end of intervention in the probiotic group (median relative change 1.05-fold, $p=0.61$). However, a statistically significant increase was observed in the placebo group from baseline to end of intervention (median relative change 1.37-fold, $p<0.01$).

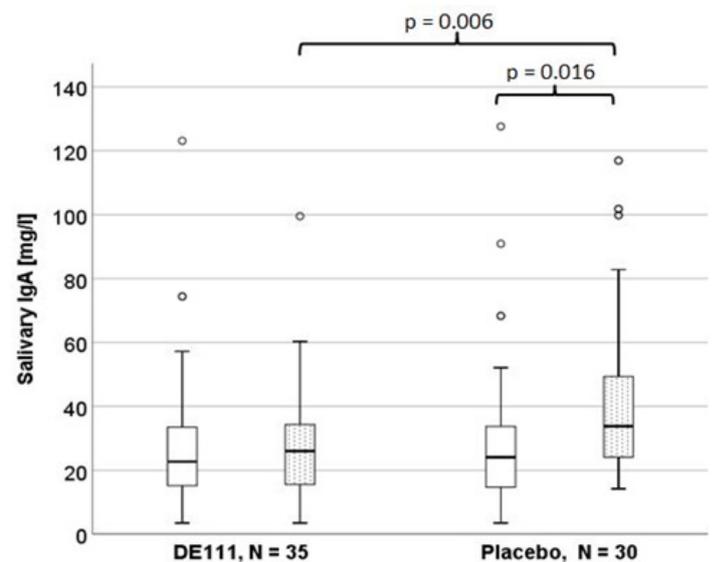


Figure 2: Boxplot of salivary IgA values at baseline (white) and after 8 weeks of intervention (dotted). Braces denote statistically significant difference. O Circles represent data outliers.

Analysis of study follow-up period

No statistically significant differences were seen between probiotic and placebo groups among any of the parameters measured in the 4-week follow-up period, in both ITT and PP analyses (Table 4). No difference in the incidence of RI was observed (28.3% probiotic vs 17% placebo, $p=0.30$). The incidence of GII was low in general throughout the 4-week follow-up (4.3% probiotic vs 0% placebo, $p=0.24$), with the duration of GII in the probiotic group totalling 5 days.

Table 4: Incidence and duration of Respiratory Infections (RI) and Gastrointestinal Infections (GII) and symptoms during the 4-week follow-up period.

Outcome	Incidence		Incidence		Duration		Duration	
	ITT population		PP population		ITT population		PP population	
	DE111®	Placebo	DE111®	Placebo	DE111®	Placebo	DE111®	Placebo
RI	13 (28.3%)	8 (17.0%)	12 (28.6%)	7 (17.9%)	130 (20)	47 (10)	119 (20)	41 (10)
GII	2 (4.3%)	0 (0%)	2 (4.8%)	0 (0%)	5 (3)	0 (0)	5 (3)	0 (0)
Diarrhoea (Bristol 6-7)	3 (6.5%)	1 (2.1%)	3 (7.1%)	0 (0%)	6 (3)	1 (1)	6 (3)	0 (0)
Hard stool (Bristol 1-2)	0 (0%)	2 (4.3%)	0 (0%)	0 (0%)	0 (0)	5 (3)	0 (0)	0 (0)
Constipation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0)	0 (0)	0 (0)	0 (0)
Bloating	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0)	0 (0)	0 (0)	0 (0)
Vomiting	1 (2.2%)	2 (4.3%)	1 (2.4%)	2 (5.1%)	1 (1)	4 (3)	1 (1)	4 (3)
Diarrhoea and vomiting	4 (8.7%)	3 (6.4%)	4 (9.5%)	2 (5.1%)	7 (3)	5 (3)	7 (3)	4 (3)
GI discomfort	3 (6.5%)	2 (4.3%)	3 (7.1%)	0 (0%)	6 (3)	6 (3)	6 (3)	0 (0)
14 (5)	14 (5)	14 (5)	14 (5)	14 (5)	14 (5)	14 (5)	14 (5)	14 (5)

DISCUSSION

Several studies have examined the role of probiotics in the prevention of GII and management of gastroenteritis with mixed results. Gastroenteritis (causing nausea, vomiting and diarrhoea) develops in millions of children every year, and treatment with probiotics may reduce the duration and intensity of symptoms [19-21]. Although the protective effects of some specific probiotic strains have been shown there is limited data to support the use of *Bacillus*-based probiotics in this specific population [22,23]. Additionally, while numerous individual symptoms have been used as outcomes, evaluations that incorporate both the duration and incidence of both diarrhoea and vomiting are lacking [24].

In this current study, the effect of the probiotic *B. subtilis* DE111® on the incidence as well as duration of GII and gastroenteritis symptoms were evaluated. While a trend of reduction in the incidence and duration of GII following probiotic intake was observed, an important drawback to note is the timing of the intervention. The peak time for GII typically falls between January and April [25]. As this study was conducted from April to July, the peak season for infections was missed, potentially impacting the incidence of GII and GI symptoms and thus limiting the evaluation of significance of probiotic intake effect. Regardless, despite low incidences of GII, the data strongly suggest a clear trend of protection and maintenance of gut health through the intake of *B. subtilis* DE111®.

In addition to support against GII, probiotics have been shown to support GI comfort. Individual indicators of GI comfort were monitored in this trial. A statistically significant decrease in the incidence of hard stools and a decrease of diarrhoea with probiotic intake was seen. When combining symptoms of GI discomfort (diarrhoea, hard stool, constipation and bloating), a statistically significant reduction of 62% in the duration of symptoms was observed in the probiotic group. Probiotics have been proposed to promote gut health through regulating gut motility and stool consistency [26]. Indeed, *B. subtilis* itself has been shown to have a

protective effect on constipation and diarrhoea in adults [15]. The results of this current study further support the role of *B. subtilis* to protect against GI discomfort in preschool aged children.

Taken together, there is an obvious trend where *B. subtilis* DE111® may have a protective role in the GI tract, supporting the maintenance of overall gut health and preventing the onset of GI disturbances in children.

The impact of probiotics against common RI in both adult and children populations has also been reviewed and evaluated in many studies with varying results on incidence and duration of RI [21,27,28]. No significant difference in the incidence or duration of RI between probiotic and placebo groups was seen in this study, however, greater than 50% reduced duration of RI was observed in the placebo group. A review of the data from this trial revealed two extreme outliers where a RI was reported for almost the entire duration of the study (46 and 36 days compared to 8 days average duration for all other children). Both outlier children were in the probiotic group, thereby impacting the overall data with respect to duration of RI. Moreover, further investigation revealed a higher number of children with recurrent RI in the preceding months in the probiotic group. This suggests a possible increased susceptibility for contracting RI in the probiotic population. While the results of this study did not indicate a protective effect of probiotic use against RI, the clear outliers in the probiotic group highlight an inherent challenge in trials conducted with healthy individuals.

The mechanisms by which probiotics modulate the immune response are not fully understood. It has been suggested that probiotics can influence both innate and adaptive immune responses by producing exopolysaccharides, causing an increase in different leukocyte populations and affecting the expression of certain interleukins such as IL-10, IL-6, and IL-8, which can contribute to an increase in sIgA [29,30]. Indeed, increases in sIgA in response to novel antigens, higher antigenic burden and following acute respiratory infections have been reported [31-33]. Interestingly, there are also reports showing the maintenance of

sIgA levels alongside improved innate immunity with probiotic intake [34,35]. In the current study, levels of sIgA from baseline to end of intervention were maintained in the probiotic group, while levels significantly increased in the placebo group by the end of the intervention. A deeper insight into sIgA oscillations during the treatment and follow-up periods would be needed to understand whether probiotic intake could support the innate immunity response.

Probiotics are generally acknowledged to act transiently in the gut, with longer term colonisation or effect typically not expected [36]. This is further supported in this study, where no significant or clinically relevant difference in GII or RI was seen between the probiotic and placebo groups in the 4-week follow up period.

CONCLUSION

In conclusion, this study suggests the probiotic *B. subtilis* DE111® has a beneficial effect in preventing GII and promoting GI health and comfort in young children attending day-care. Additionally, it shows the probiotic is well tolerated and safe to use in children 2-6 years old. Future studies would benefit from interventions, during peak infection season.

CONFLICT OF INTEREST

Dr AM Winger is an employee of Deerland Probiotics and Enzymes, however, was not involved in the execution of the trial, data management or statistical analysis.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing of the manuscript. MS and KČK were involved in protocol writing, study execution and data management. RO was the medical advisor for the study and reviewed the manuscript. NČL and IL performed the statistical analysis of the study. AW prepared and reviewed the manuscript.

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REFERENCES

1. Enserink R, Ypma R, Donker GA, Smit HA, van Pelt W. Infectious disease burden related to child day care in the Netherlands. *Pediatr Infect Dis J*. 2013;32(8):e334-340.
2. Nafstad PHJ, Oie L, Megnus P, Jaakkola JJK. Day care centres and respiratory health. *Pediatrics*. 1999;103(4):753-758.
3. Louhiala PJJN, Ruotsalainen R, Jaakkola JJK. Day-care centers and diarrhea: A public health perspective. *The Journal of Pediatrics*. 1997;131(3):476-479.
4. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*. 2014;11(8):506-514.
5. 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(2):e172-179.
6. Hatakka K, Savilahti E, Pönkä A, Meurman JH, Poussa T, Näse L, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: Double blind, randomised trial. *BMJ*. 2001;322:1327.
7. Wallace B. Clinical use of probiotics in the pediatric population. *Nutrition in Clinical Practice*. 2009;24(1):50-59.
8. 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(1):5-9.
9. Laursen RP, Hojsak I. Probiotics for respiratory tract infections in children attending day care centers-a systematic review. *Eur J Pediatr*. 2018;177(7):979-994.
10. Hojsak I. Probiotics in Children: What Is the Evidence? *Pediatr Gastroenterol Hepatol Nutr*. 2017;20(3):139-146.
11. Hong HA, Khaneja R, Tam NM, Cazzato A, Tan S, Urdaci M, et al. *Bacillus subtilis* isolated from the human gastrointestinal tract. *Res Microbiol*. 2009;160(2):134-143.
12. Ellis-Pegler RB, Crabtree C, Lambert HP. The faecal flora of children in the United Kingdom. *J Hyg (Lond)*. 1975;75(1):135-142.
13. Lakshmi SG, Jayanthi N, Saravanan M, Ratna MS. Safety assessment of *Bacillus clausii* UBBC07, a spore forming probiotic. *Toxicol Rep*. 2017;4:62-71.
14. Horosheva TV, Sorokulova I, Vodyanov V. Efficacy of *Bacillus* probiotics in prevention of antibiotic-associated diarrhoea: A randomized, double-blind, placebo-controlled clinical trial. *JMM Case Reports*. 2014;1(3).
15. Cuentas AM, Deaton J, Khan S, Davidson J, Ardita C. The effect of *Bacillus subtilis* DE111 on the daily bowel movement profile for people with occasional gastrointestinal irregularity. *J Prob Health*. 2017;5(4).
16. Toohey JC, Townsend JR, Johnson SB, Toy AM, Vantrease WC, Bender D, et al. Effects of probiotic (*Bacillus subtilis*) supplementation during offseason resistance training in female division I athletes. *The J Strength Conditioning Res*. 2018.
17. Labellarte G, Cooper S, Maher M. Tolerance and efficacy of a probiotic supplement delivered in capsule form. *The FASEB Journal*. 2015;29(1):924-933.
18. Paytuyi-Gallart A, Sanseverino W, Winger AM. Daily intake of probiotic strain *Bacillus subtilis* DE111 supports a healthy microbiome in children attending day-care. *Benef Microbes*. 2020;11(7):611-620.
19. Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, et al. Use of probiotics for management of acute gastroenteritis: A position paper by the ESPGHAN working group for probiotics and prebiotics. *J Pedi Gastroenterology Nutri*. 2014;58(4):531-539.
20. Schnadower D, Tarr PI, Casper TC, Gorelick MH, Dean JM, O'Connell KJ, et al. *Lactobacillus rhamnosus* GG versus placebo for acute gastroenteritis in children. *New England J Med*. 2018;379(21):2002-2014.
21. Hojsak I, Snovak N, Abdovic 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(3):312-316.
22. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. *Lancet Infect Dis*. 2006;6(6):374-382.
23. Patro-Gołąb B, Szajewska H. Systematic review with meta-analysis: *Lactobacillus reuteri* DSM 17938 for treating acute gastroenteritis in children. *An Update. Nutrients*. 2019;11(11):2762.
24. Freedman SB, Williamson-Urquhart S, Farion KJ, Gouin S, Willan AR, Poonai N, et al. Multicenter trial of a combination probiotic for children with gastroenteritis. *New England J*

- Medi. 2018;379(21):2015-2026.
25. Maja Solla KPT, Nataša Berginc, Saša Steiner Rihtar. Influenza and acute respiratory infection season in slovenia 2018/2019 Ljubljana, Slovenia. 2019.
 26. Yoon JY, Cha JM, Oh JK, Tan PL, Kim SH, Kwak MS, et al. Probiotics ameliorate stool consistency in patients with chronic constipation: A randomized, double-blind, placebo-controlled study. *Dig Dis Sci.* 2018;63(10):2754-2764.
 27. Hao Q, Dong BR, Wu T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev.* 2015;2:Cd006895.
 28. Vouloumanou EK, Makris GC, Karageorgopoulos DE, Falagas ME. Probiotics for the prevention of respiratory tract infections: A systematic review. *Int J Antimicrob Agents.* 2009;34(3):e1-10.
 29. Kawashima T, Ikari N, Kouchi T, Kowatari Y, Kubota Y, Shimojo N, et al. The molecular mechanism for activating IgA production by *Pediococcus acidilactici* K15 and the clinical impact in a randomized trial. *Sci Rep.* 2018;8(1):5065.
 30. Ahrne S, Hagslatt M-LJ. Effect of lactobacilli on paracellular permeability in the gut. *Nutrients.* 2011;3(1):104-117.
 31. Gleeson M, Dobson AJ, Firman DW, Cripps AW, Clancy RL, Włodarczyk JH, et al. The variability of immunoglobulins and albumin in salivary secretions of children. *Scand J Immunol.* 1991;33(5):533-541.
 32. Gleeson M, Cripps AW, Clancy RL. Modifiers of the human mucosal immune system. *Immunol Cell Biol.* 1995;73(5):397-404.
 33. Mellander L, Carlsson B, Hanson LA. Appearance of secretory IgM and IgA antibodies to *Escherichia coli* in saliva during early infancy and childhood. *J Pediatr.* 1984;104(4):564-568.
 34. Berman SH, Eichelsdoerfer P, Yim D, Elmer GW, Wenner CA. Daily ingestion of a nutritional probiotic supplement enhances innate immune function in healthy adults. *Nutrition Research.* 2006;26(9):454-459.
 35. Cox AJ, Pyne DB, Saunders PU, Fricker PA. Oral administration of the probiotic *Lactobacillus fermentum* VRI-003 and mucosal immunity in endurance athletes. *British J Sports Med.* 2010;44(4):222-226.
 36. Ciorba MA. A gastroenterologist's guide to probiotics. *Clin Gastroenterol Hepatol.* 2012;10(9):960-968.