Case Report

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Efficacy of *Bacillus* probiotics in prevention of antibiotic-associated diarrhoea: a randomized, double-blind, placebo-controlled clinical trial

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Introduction: Antibiotic-associated diarrhoea (AAD) is one of the most common side effects of antibiotic therapy. The main mechanism associated with the development of AAD is significant changes in the composition and quantity of the gut microbiota during the treatment with antibiotics. Probiotic bacteria have been shown to stabilize the gut microbiota and can be used to prevent diarrhoea associated with antibiotic therapy.

Case presentation: We present the results of a single-centre, randomized, double-blinded, placebo-controlled clinical trial. Patients were randomized into three groups: probiotic group 1 received a probiotic containing strains *Bacillus subtilis* 3 and *Bacillus licheniformis* 31; probiotic group 2 received a probiotic, containing *B. subtilis* 3; and the placebo group received an inert composition in vials, formulated to be indistinguishable from the vials with probiotics. Participants received one vial twice a day. Probiotic treatment significantly reduced incidents of AAD in the patients. Among 91 patients in group 1 treated with probiotic mix, nine developed AAD. In group 2, seven patients out of 90 who received only one probiotic strain developed AAD. A considerably higher incidence of AAD was registered in the placebo group – 23 from 90 patients ($P < 0.001$ vs groups 1 and 2). Both probiotics demonstrated a significant effect in the prevention of nausea, bloating, vomiting and abdominal pain.

Conclusion: Treatment with *Bacillus* probiotics during antibiotic therapy significantly decreased the incidence of AAD and adverse effects related to the use of antibiotics. Both probiotics were well tolerated by the patients without side effects. No significant difference was found in the efficacy of the two probiotics.

Keywords: abdominal symptoms; antibiotic-associated diarrhoea; *Bacillus* probiotics.

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Introduction

One of the most common side effects of antibiotic therapy is antibiotic-associated diarrhoea (AAD). The frequency of AAD depends on the antibiotic used and varies from 2 to 25 % (Bartlett, 2002) and can be as high as 44 % (Gao *et al.*, 2010). The route of antibiotic administration (oral or parenteral) does not affect the rate of AAD (Bartlett, 2002), and no difference has been found in frequency of AAD with respect to age and gender (Wistrom *et al.*, 2001). The severity of AAD may vary from uncomplicated diarrhoea to *Clostridium difficile*-associated pseudomembranous colitis. AAD may be caused by different enteric pathogens (e.g. *Salmonella* spp., *Staphylococcus aureus*, *Candida albicans*, *Clostridium perfringens* and *Klebsiella* spp.) (Bartlett, 2002; Gorkiewicz, 2009). The main mechanism

for the development of AAD is significant changes in the composition and quantity of the gut microbiota during the treatment with antibiotics (Young and Schmidt, 2004). Probiotic bacteria have been shown to stabilize the gut microbiota and to prevent diarrhoea associated with antibiotic use (Friedman, 2012; Persborn *et al.*, 2013). *Bacillus* bacteria have attracted the growing attention of researchers as effective probiotics for the prevention and treatment of enteric infections (Coppi *et al.*, 1985; Canani *et al.*, 2007; Mazza, 1994). Previously, we showed a high efficacy of the *Bacillus* probiotic Biosporin in the treatment of acute intestinal infections (Gracheva *et al.*, 1996). Biosporin is a mix of two strains – *B. subtilis* 3 and *B. licheniformis* 31, with a predominant amount of *B. subtilis* 3 (50:1). These strains have been deposited in the Russian National Collection of Industrial Micro-organisms (VKPM) as *B. subtilis* B2335 and *B. licheniformis* B2336. Both *Bacillus* strains have been thoroughly tested in pre-clinical testing (Pinchuk *et al.*, 2001; Sorokulova, 2008;

Abbreviations: AAD, antibiotic-associated diarrhoea; ARR, absolute risk reduction; CI, confidence interval; CDD, *Clostridium difficile*-associated diarrhoea; OR, odds ratio; RR, relative risk

Sorokulova *et al.*, 1997), including safety evaluations (Sorokulova *et al.*, 2008), and in clinical trials (Gracheva *et al.*, 1996; Sorokulova *et al.*, 2003). Biosporin is approved by the National Control Authority for Biological Products of Ukraine and Russia as a drug for adults and children. Further studies of this probiotic showed that *B. subtilis* 3 had a higher antagonistic activity against pathogens than *B. licheniformis* 31 (Pinchuk *et al.*, 2001; Sorokulova *et al.*, 1997).

Our hypothesis was that *Bacillus* probiotics would reduce the frequency of AAD in patients treated with antibiotics. We also postulated that the efficacy of one *B. subtilis* 3 strain with pronounced antagonistic activity against pathogens would be the same as the efficacy of a two-probiotic culture (*B. subtilis* 3 and *B. licheniformis* 31).

Case report

The main objectives of this study were to analyse efficacy of *Bacillus* probiotics in the prevention of AAD and to compare the efficacy of two *Bacillus* probiotics in a single-centre, randomized, double-blinded, placebo-controlled clinical trial. The study was conducted at the Medical Center Vitbiomed OOO in Moscow, Russia, from October 2011 to November 2012. The study protocol was approved by the internal review board of the centre. Written informed consent was obtained from each patient. Adult outpatients aged 45 years or over who were anticipated to take one or more oral or intravenous antibiotics for at least 5 days were recruited for this trial.

Exclusion criteria were existing diarrhoea, *Clostridium difficile*-associated diarrhoea (CDD) within the previous 3 months, use of probiotics in the previous 3 weeks, adverse reactions to microbial preparations in the past and immunocompromised patients. Patients were assigned to each group by simple randomization procedures (computer-generated random numbers).

Each patient received a coded reporting form to record clinical data. Patients were instructed to record daily the number of faecal outputs and their consistency, and the presence of vomiting, nausea, abdominal pain, bloating and any other adverse events.

Investigation and treatment

Patients were randomized into three groups: (i) probiotic group 1, who received a probiotic, containing strains *B. subtilis* 3 and *B. licheniformis* 31 (2×10^9 c.f.u. per vial); (ii) probiotic group 2, who received a probiotic containing *B. subtilis* 3 (2×10^9 c.f.u. per vial); and (iii) a placebo group, who received an inert composition in vials, formulated to be indistinguishable from the vials with probiotics.

Maltodextrin was used as an inert component in all vials (probiotics and placebo). The volume of all vials was 2 ml. The probiotic vials did not vary from placebo in appearance,

colour, taste or size. All vials were prepared by Vitbiomed OOO by independent production staff who did not have patient contact or data management responsibilities.

Bacterial cultures were grown on agar medium and harvested with sterile PBS. The number of live bacteria (c.f.u.) was identified by plating of 10-fold dilutions of bacterial suspension on nutrient agar plates. The bacterial suspension was diluted with sterile PBS to obtain 1×10^9 c.f.u. ml⁻¹. This suspension was dispensed into opaque plastic vials in 2 ml volumes to obtain 2×10^9 c.f.u. in each vial.

The study was conducted as a double-blinded clinical trial. The allocation sequence was performed by independent personnel and was unavailable to members of the research team and patients until data analysis had been completed. Patients, clinical staff and biostatisticians were blinded to treatment allocation throughout the trial.

The assigned intervention started 1 day before the beginning of antibiotic therapy and continued for 7 days after discontinuation of antibiotics. Participants received one vial (2 ml dose) in the morning and one in the evening prior to their meals. Patients were followed up for 4 weeks after stopping the antibiotics, unless AAD occurred before that time.

The primary outcomes were the incidence of AAD during the study period. The diarrhoea was defined as three or more loose or watery stools day⁻¹ for at least 2 days. In the case of diarrhoea, a stool sample was collected and analysed for the presence of *Clostridium difficile* toxins by an immunoenzymatic assay (*C. difficile* TOX A/B II; TechLab).

Secondary outcomes included the occurrence of CDD, abdominal symptoms, adverse effects and the acceptability of the probiotics.

In order to achieve a statistical power of 85 % at a 5 % significance level, we estimated the sample size of this trial to be $n=270$ (90 per group), allowing for a maximum 10 % dropout rate in each group. A χ^2 test was used to compare all three groups. The χ^2 test with Bonferroni correction [α (0.05)/number of tests ($n=3$)] was applied for further pairwise comparison. Additionally, we calculated the relative risk (RR) and odds ratio (OR) together with their 95 % confidence interval (CI) for each of the characteristics. We also calculated the absolute risk reduction (ARR) when comparing treatment groups. Continuous variables were summarized using (i) the number of observations; (ii) the median and interquartile range; or (iii) the mean and SD.

Outcomes

The patient flow is summarized in Fig. 1. In all, 574 patients were screened for participation and 308 were enrolled in the study. The major reasons for exclusion were unwillingness to participate (221, 38.5 %), existing

Fig. 1. Patients flow diagram.

diarrhoea (15, 2.6 %), use of probiotics in the previous 3 weeks (19, 3.3 %) and immunocompromised patients (11, 1.9 %). Follow-up was not completed for 37 (12 %) patients because of loss of contact (23, 7.5 %) or because they had withdrawn from the study (14, 4.5 %) (Fig. 1). Thus, analysis was performed for 271 patients ($n=91$ for probiotic mix, $n=90$ for probiotic and $n=90$ for placebo).

There was no difference in baseline characteristics of patients in the three groups (Table 1). Patients in all three groups were matched for gender and age. The indications for treatment with antibiotics were similar in all groups with a prevalence of respiratory and urinary tract infections (Table 1). The antibiotic regimen exposure was similar among the three groups.

Probiotic treatment significantly reduced the incidence of AAD in patients. Thus, among 91 patients in group 1 treated with the probiotic mix, nine (9.9 %) patients developed AAD (Table 1). In group 2, seven patients (7.8 %) of the 90 receiving only one probiotic strain developed AAD. No difference was found between probiotic groups 1 and 2 in the prevention of AAD ($P=0.617$; $RR=1.272$, 95 % CI 0.495–3.268; $OR=1.301$,

95 % CI 0.463–3.659). A considerably higher incidence of AAD was registered in the placebo group, in 23 (32.2 %) of the 90 patients ($P<0.001$ vs groups 1 and 2; $RR=0.307$, 95 % CI 0.154–0.611 vs group 1; $RR=0.241$, 95 % CI 0.112–0.522 vs group 2; ORs were similar) (Table 2). The ARR for occurrence of AAD for group 1 was 22 % (95 % CI 11–34 %) and the number needed to treat was 4 (95 % CI 3–9). Similar data were obtained for group 2: $ARR=24$ % (95 % CI 13–37 %) and the number needed to treat was 4 (95 % CI 3–8). No cases of CDD were detected in any of the groups.

Analysis of morbidity in the study groups showed a significant effect of probiotic treatment in the prevention of nausea, bloating, vomiting and abdominal pain (Table 1). Only one patient (1.1 %) in probiotic group 1 reported the development of nausea in comparison with nine (10 %) patients in the placebo group ($P=0.009$; $RR=0.11$, 95 % CI 0.014–0.850; $OR=0.1$, 95 % CI 0.012–0.807) (Table 2). No vomiting was registered in the probiotic groups, whilst in the placebo group 11 (12.2 %) patients had vomiting ($P=0.001$). Incidents of bloating were registered in eight (8.8 %) patients in probiotic group 1 and seven (7.8 %) in probiotic group 2 ($P=0.805$;

Table 1. Baseline characteristics of patients and outcomes

Characteristics	Group		
	Probiotic mix (group 1) (n=91)	Probiotic (group 2) (n=90)	Placebo (group 3) (n=90)
Baseline characteristics of patients			
Age (years)	55.6 ± 5.9	56.8 ± 5.8	56.1 ± 5.4
Gender			
Male	45 (49 %)	48 (53 %)	47 (52 %)
Female	46 (51 %)	42 (47 %)	43 (48 %)
Diagnosis for antibiotic treatment			
Respiratory tract infections	44 (48.3 %)	43 (47.8 %)	46 (51.1 %)
Urinary tract infections	34 (37.4 %)	35 (38.9 %)	33 (36.7 %)
Other*	13 (14.3 %)	12 (13.3 %)	11 (12.2 %)
Co-morbidity			
Asthma	12 (13.2 %)	10 (11.1 %)	13 (14.4 %)
Cardiovascular disease	9 (9.9 %)	11 (12.2 %)	14 (15.5 %)
Gastritis	7 (7.7 %)	6 (6.7 %)	4 (4.4 %)
Malignancy	5 (5.5 %)	3 (3.3 %)	6 (6.7 %)
Diabetes mellitus	15 (16.5 %)	13 (14.4 %)	12 (13.3 %)
Antibiotic therapy			
Broad-spectrum penicillins	46 (50.5 %)	49 (54.4 %)	48 (53.3 %)
Cephalosporins	36 (39.6 %)	33 (36.7 %)	35 (38.9 %)
Macrolides	9 (9.9 %)	8 (8.9 %)	7 (7.8 %)
Duration of antibiotic therapy (days)	7.4 ± 1.7	7.3 ± 1.8	7.6 ± 1.7
Outcomes			
AAD†	9 (9.9 %)	7 (7.8 %)	29 (32.2 %)
<i>Clostridium difficile</i> diarrhoea	0	0	0
Morbidity			
Nausea	1 (1.1 %)	0	9 (10 %)
Vomiting	0	0	11 (12.2 %)
Bloating	8 (8.8 %)	7 (7.8 %)	26 (28.9 %)
Abdominal pain	10 (10.9 %)	8 (8.9 %)	32 (35.5 %)

*Prophylaxis after surgery, skin infections. †Data presented as number of patients, n (%).

RR=1.13, 95 % CI 0.428–2.987; OR=1.143, 95 % CI 0.396–3.295). A significantly higher number of patients (26, 28.9 %) with bloating were indicated in the placebo group ($P<0.001$ vs both probiotic groups; RR=0.304, 95 % CI 0.146–0.636 vs group 1; RR=0.269, 95 % CI 0.123–0.588 vs group 2; ORs were similar) (Table 2). Abdominal pain was reported by 10 (10.9 %) patients in group 1 and eight (8.9 %) in group 2 ($P=0.637$; RR=1.236, 95 % CI 0.511–2.989; OR was similar) (Table 2). Patients in the placebo group had a significantly higher incidence of abdominal pain (32, 35.5 %) ($P<0.001$ vs both probiotic groups; RR=0.309, 95 % CI 0.162–0.591 vs group 1; RR=0.250, 95 % CI 0.122–0.512 vs group 2; ORs were similar) (Table 2).

Both probiotics were well tolerated and no probiotic-related adverse events were noted.

Discussion

The results of our study showed the efficacy of *Bacillus* probiotics in prevention of AAD during treatment with antibiotics. Beneficial effects of probiotics were also found in significant mitigation of secondary outcomes: abdominal

pain, nausea, bloating and vomiting. These findings are in accordance with our previous data about the high efficacy of *Bacillus* probiotic in the elimination of abdominal symptoms in patients with acute intestinal infections (Gracheva *et al.*, 1996). This study demonstrated that the efficacy of a single-strain probiotic (*B. subtilis* 3) was the same as a mix of two *Bacillus* strains (*B. subtilis* 3 and *B. licheniformis* 31). The therapeutic success of probiotics depends on a variety of beneficial effects that are unique for each probiotic strain. We can assume that the efficacy of both tested probiotics was determined by the high probiotic activity of *B. subtilis* 3 strain, as indicated *in vitro* (Pinchuk *et al.*, 2001; Sorokulova *et al.*, 1997). To the best of our knowledge, this is the first report on the clinical efficacy of *B. subtilis* probiotic in the prevention of AAD. Previously, the *Bacillus clausii* probiotic strain was found to be effective in the reduction of diarrhoea incidents related to anti-*Helicobacter pylori* antibiotic therapy (Nista *et al.*, 2004). Another probiotic strain, *Bacillus coagulans* GBI-30, 6086, was effective in decreasing abdominal pain and bloating symptoms in patients with inflammatory bowel disease (Hun, 2009) and in reducing daily bowel movements in patients with irritable bowel syndrome (Dolin, 2009). In patients with post-prandial gas-related

Table 2. Statistical analysis of outcomes

Characteristic	G1 vs G2	OR (95 % CI)	G1/G2	RR (95 % CI)	ARR (G2-G1)	G1 vs G3	OR (95 % CI)	G1/G3	RR (95 % CI)	ARR (G3-G1)	G2 vs G3	OR (95 % CI)	G2/G3	RR (95 % CI)	ARR (G3-G2)
Diarrhoea	$P=0.617$	1.301 (0.463–3.659)	–	1.272 (0.495–3.268)	0.02 (–0.061–0.104)	$P<0.001$	0.231 (0.102–0.523)	–	0.307 (0.154–0.611)	0.22 (0.109–0.338)	$P<0.001$	0.177 (0.073–0.432)	–	0.241 (0.112–0.522)	0.24 (0.133–0.356)
CDD	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nausea	$P=0.319$	–	–	–	0.01 (–0.010, 0.032)	$P=0.009$	0.1 (0.012, 0.807)	Morbidity	0.110 (0.014, 0.850)	0.09 (0.023, 0.155)	$P=0.002$	–	–	–	0.10 (0.0380.162)
Vomiting	–	–	–	–	–	$P=0.001$	–	–	–	0.12 (0.055, 0.190)	$P=0.001$	–	–	–	0.12 (0.055, 0.190)
Bloating	$P=0.805$	1.143 (0.396, 3.295)	–	1.130 (0.428, 2.987)	0.01 (–0.070, 0.090)	$P<0.001$	0.237 (0.101, 0.559)	–	0.304 (0.146, 0.636)	0.20 (0.091, 0.311)	$P<0.001$	0.208 (0.085, 0.509)	–	0.269 (0.123, 0.588)	0.21 (0.102, 0.320)
Abdominal pain	$P=0.637$	1.265 (0.475, 3.369)	–	1.236 (0.511, 2.989)	0.02 (–0.066, 0.108)	$P<0.001$	0.224 (0.102, 0.491)	–	0.309 (0.162, 0.591)	0.25 (0.128, 0.364)	$P<0.001$	0.177 (0.076, 0.411)	–	0.250 (0.122, 0.512)	0.27 (0.152, 0.382)

G1, group 1 (two probiotic strains, *B. subtilis* 3 and *B. licheniformis* 31); G2, group 2 (one probiotic strain *B. subtilis* 3); G3, group 3 (placebo).

symptoms, *B. coagulans* GBI-30, 6086 probiotic strain was effective in improving the quality of life and reducing gastrointestinal symptoms (abdominal pain, abdominal distention and flatus) (Kalman *et al.*, 2009). The mechanism of the beneficial effects of *Bacillus* probiotics on the gastrointestinal tract is not completely known, but some properties known for *Bacillus* bacteria may contribute to their efficacy. *Bacillus* bacteria are metabolically highly active and they produce anti-microbial substances, amino acids and vitamins (Sorokulova, 2008). They could support digestive function of the gut by producing essential enzymes (proteolytic, lipolytic or cellulolytic). *Bacillus* bacteria may provide the host with the ability to maintain intestinal homeostasis. The quorum-sensing pentapeptide of *B. subtilis*, competence and sporulation factor, activates key survival pathways in intestinal epithelial cells of the host (Fujiya *et al.*, 2007). It was shown that this competence and sporulation factor induces heat-shock proteins, which protect intestinal epithelial cells against injury and loss of barrier function. Clinical efficacy of *Bacillus* bacteria in the treatment of gastrointestinal infections has been reported by authors from different countries. Mazza (1994) summarized the main results of these studies and concluded that *B. subtilis* is one of the most important micro-organisms for the therapy and prophylaxis of intestinal disorders in humans. In clinical trials, *Bacillus* probiotic was more effective in the treatment of acute intestinal infections than *Lactobacillus* probiotic (Gracheva *et al.*, 1996).

Our study demonstrated that treatment with *Bacillus* probiotics during antibiotic therapy significantly decreased the incidence of AAD and adverse effects related to antibiotics. Both probiotics were well tolerated by the patients without side effects. No significant difference was found in the efficacy of one probiotic strain (*B. subtilis* 3) in comparison with a mix of two strains (*B. subtilis* 3 and *B. licheniformis* 31).

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