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MAJOR ARTICLE

High-dose Probiotic Mix of *Lactobacillus* spp., *Bifidobacterium* spp., *Bacillus coagulans*, and *Saccharomyces boulardii* to Prevent Antibiotic-associated Diarrhea in Adults: A Multicenter, Randomized, Double-blind, Placebo-controlled Trial (SPAADA)

Vladimir Hodzhev,[1] Karen Dzhambazov,[1] Nikolay Sapundziev,[2] Milena Encheva,[3] Spiridon Todorov,[4] Vania Youroukova,[5] Rumen Benchev,[6] Rosen Nikolov,[7] Boris Bogov,[8] Georgi Momekov,[9] and Veselin Hadjiev[10]

[1]University Hospital for Active Treatment "Sveti Georgi" JSC, Medical University of Plovdiv, Plovdiv, Bulgaria, [2]Department of Neurosurgery and Otorhinolaryngology, Medical University of Varna, Varna, Bulgaria, [3]Military Medical Academy, Sofia, Bulgaria, [4]University Hospital "Tsaritsa Yoanna-ISUL", Medical University of Sofia, Sofia, Bulgaria, [5]Clinic for the treatment of nonspecific pulmonary diseases and physiatry, UMHAT "St. Ivan Rilski", Medical University of Sofia, Sofia, Bulgaria, [6]Hill Clinic, Sofia, Bulgaria, [7]UMHAT "St. Ivan Rilski", Medical University of Sofia, Sofia, Bulgaria, [8]UMHAT "St. Anna" Department of Internal Diseases, Medical University of Sofia, Sofia, Bulgaria, [9]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria, and [10]Research Institute, University of Economics, Varna, Bulgaria

Background. Probiotics have been used to prevent antibiotic-associated diarrhea (AAD), but practical guidelines are sparse. This trial evaluated the efficacy and safety of a high-dose, multistrain probiotic mix (Sinquanon), specially designed for prevention of AAD in adults.

Methods. A phase IV, multicenter, randomized, double-blind, placebo-controlled, parallel-group clinical trial was conducted over 5 months. Participants receiving broad-spectrum antibiotics were administered the specialized probiotic mix or placebo from the first dose of antibiotics until 14 days after the last antibiotic dose. The primary outcome measure was the incidence of AAD.

Results. In total, 564 participants were randomized (probiotic mix: 285; placebo: 279), of which 9 participants discontinued the trial early (probiotic mix: 3; placebo: 6), had no efficacy data, and were excluded from the efficacy analysis. The 555 remaining participants completed the trial and were included in the efficacy analysis (probiotic mix: 282; placebo: 273). AAD occurred less frequently in the studied probiotic mix versus placebo group (9.2% vs 25.3%, $P < .001$), resulting in an absolute risk reduction of 16% and a number needed to treat of 6 (95% confidence interval, 4.55–10.49). A significant improvement in the average gastrointestinal quality of life in the studied probiotic mix versus placebo group was also observed. There were no clinically relevant differences in the incidence of adverse events between the studied probiotic mix and the placebo group.

Conclusions. The specially designed high-dose, multistrain probiotic mix (Sinquanon) demonstrated to be beneficial compared with placebo in the prevention of AAD in adults who received broad–spectrum antibiotics.

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Correspondence: Georgi Momekov, PhD, DSc, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria (gmomekov@pharmfac.mu-sofia.bg).

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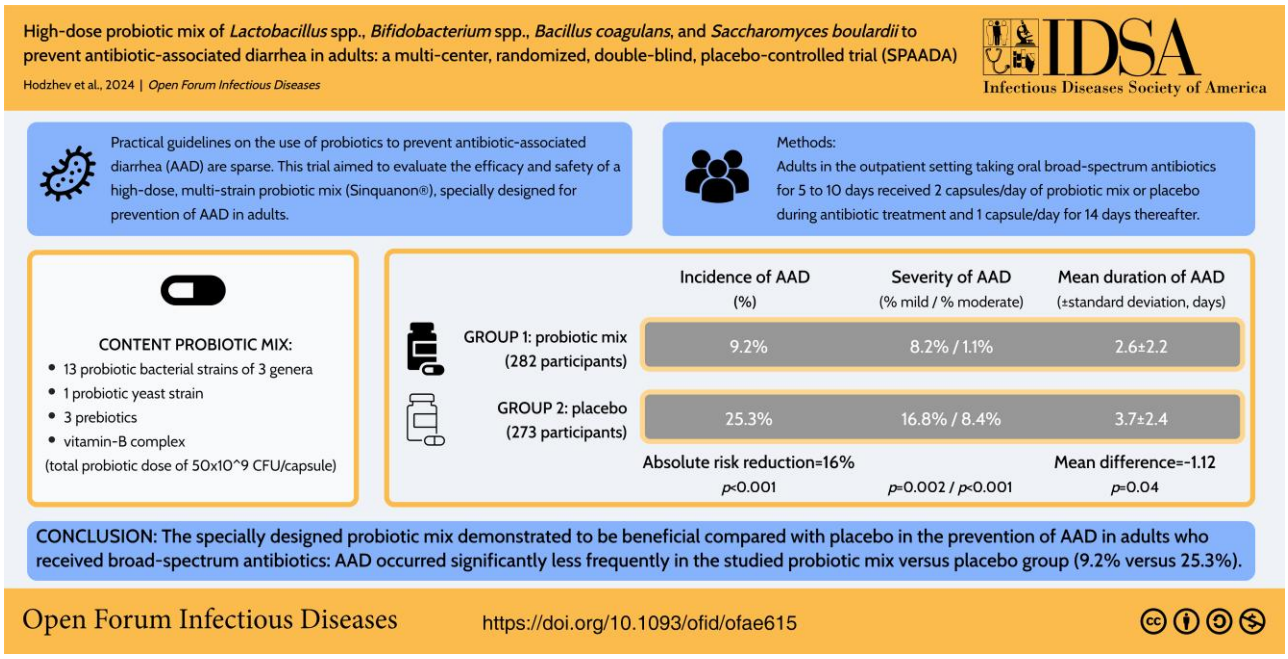
Graphical Abstract



Keywords. antibiotic-associated diarrhea; high-dose; multi-strain; probiotic; randomized controlled trial.

Gut microbiota is essential for normal gut functioning, assisting in digestion and absorption, but also immune defense and homeostasis [1]. Antibiotic treatment disrupts this beneficial, symbiotic relationship by inducing acute microbiota alterations resulting in an increased susceptibility to colonization of opportunistic pathogens, such as *Clostridioides difficile* and to antibiotic-associated diarrhea (AAD) [2].

AAD can occur during antibiotic treatment and up to 8 weeks after treatment stop. Typically, 5% to 35% of patients taking antibiotics develop AAD, depending on the type of antibiotic administered, the initial health of the host, and exposure to opportunistic pathogens [3]. Although each antibiotic may cause AAD, broad-spectrum antibiotics that primarily target anaerobes and are poorly absorbed (like cephalosporins, aminopenicillins, combination of aminopenicillins and clavulanate) bear a higher risk of AAD [4, 5].

Probiotics have been used to prevent AAD. Different meta-analyses have indicated the potential benefits of specific probiotic strains in the prevention of AAD both in children and adults. These include the yeast species *Saccharomyces boulardii* and the bacterial strain *Lactobacillus rhamnosus* GG [6, 7]. Despite the moderate quality of evidence, both are among the probiotic strains with the highest level of recommendation in the guidelines for probiotic use in the prevention of AAD published by leading expert organizations, including the World Gastroenterology Organisation and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Working Group for Probiotics/Prebiotics [8–10]. However, at present there is no global consensus for the clinical use of probiotics for AAD, and practical guidelines are sparse. Further research is needed to help clinicians make evidence-based choices on probiotic use for treating AAD [11–13].

The probiotic mix (Sinquanon) studied in the current clinical trial was developed specifically for patients on antibiotic therapy and includes the aforementioned *S boulardii and L rhamnosus* GG. In addition, 6 other *Lactobacillus* spp. and 5 *Bifidobacterium* spp., lactic-acid producing bacteria generally known for their beneficial symbiotic characteristics [14], were included in the studied probiotic mix, as well as *Bacillus coagulans*. The latter bacteria are known to withstand adverse conditions by transforming into spores on which antibiotics have limited effect. They also reduce the colonization of vancomycin-resistant enterococci [15] and secrete coagulin and lactic acid that have an inhibitory effect on pathogens [16, 17]. In total, the studied probiotic mix contains 14 different probiotic strains from 4 selected genera (see Supplementary Appendix Table 1 for the exact composition) because it has been shown that a blend of several strains showed greater efficacy than the use of a single strain [18]. The individual strains were selected for intrinsic resilience and/or resistance to antibiotics to increase the chances of strain survival and ecosystem restoration, so that regardless of the antibiotic class administered some strains survive. Note that *S boulardii* is unaffected by antibiotics but sensitive to antifungals because it is a yeast. Moreover, the different strains were selected

to produce various bacteriocins to promote synergism in the suppression of opportunistic pathogens that may be activated after antibiotic therapy [19]. The strains were also selected for interbacterial interactions important for metabolism and survival.

Dose-response studies have indicated a positive correlation between the dose of the probiotic administered and a reduced risk of AAD, with a dose greater than 10^{10} colony-forming units (CFU) being most effective [20, 21]. Therefore, the studied probiotic mix contains a high number of live bacteria and yeast (ie, 100×10^9 CFU per daily dose), allowing faster ecosystem recovery.

In addition, the studied probiotic mix contains a prebiotic blend and a vitamin B complex (see Supplementary Appendix Table 1 for additional details). The prebiotic blend of fructo-, malto-, and xylo-oligosaccharides is included to improve the survival of the selected strains and give them an advantage in the replication process because these saccharides are known to be selectively fermented by beneficial bacteria commensal to the colon (such as lactobacilli and bifidobacteria) [22]. The vitamin B complex is included to support the energy metabolism of the selected strains because B vitamins are actively involved in the metabolism of carbohydrates in the bacterial cell and bacteria use them as a source of energy [23]. The presence of B vitamins is furthermore hypothesized to help the strains divide faster and produce greater amounts of lactic and acetic acid, thereby decreasing the time to restoring the natural gut environment and increasing the inhibitory effect against pathogens [19].

The aim of this trial was to evaluate the efficacy and safety of the high-dose, multistrain probiotic mix, Sinquanon, for AAD prevention in adults in outpatient medical practice.

MATERIALS AND METHODS

Trial Design

A phase IV, multicenter, randomized, double-blind, placebo-controlled, parallel-group clinical trial (NCT05607056) was conducted between November 2022 and April 2023 in 63 outpatient pulmonology and ear, nose, and throat (ENT) practices in Bulgaria. The protocol and informed consent form were reviewed and approved by an appropriate ethics committee before trial initiation. The trial was conducted according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guidelines and the Declaration of Helsinki and was completed per protocol.

Trial Population

Key inclusion criteria for participant eligibility were adults who initiated oral antibiotic treatment in the ambulatory setting, consisting of 1 or 2 antibiotics (ie, broad-spectrum penicillins, cephalosporins, quinolones, and tetracyclines) with a total duration of 5 to 10 days.

Key exclusion criteria included antibiotics use within 60 days before randomization; daily consumption of probiotics, yogurt with probiotics, and inability to stop this consumption; an episode of diarrhea within 30 days before screening; and prior infection with *C difficile* ≤3 months before screening.

A list of all criteria can be found in the Supplementary Appendix. All participants provided a signed informed consent before their participation in the trial.

Study Schedule

Each participant was monitored for 26 to 31 days including 4 scheduled study visits:

- Visit 1 (on day 1, day of first antibiotic dose): enrollment, randomization, allocation to the studied probiotic mix/placebo, and administration of studied probiotic mix/placebo in addition to the prescribed antibiotic treatment for 5 to 10 days.
- Visit 2 (1 ± 2 days after the last antibiotic dose): completion of the antibiotic treatment and administration of the studied probiotic mix/placebo alone for 14 days after the last antibiotic dose.
- Visit 3 (14 ± 2 days after the last antibiotic dose): completion of studied probiotic mix/placebo treatment.
- Telephone call visit 4 (21 ± 2 days after the last antibiotic dose): follow-up by phone 1 week after the last dose of studied probiotic mix/placebo.

Randomization and Blinding

The randomization software Randomsamp (Randomsamp Software, Varna, Bulgaria) assigned the participants to the studied probiotic mix/placebo group (1:1, stratified by center). The trial interventions were prepared on site by Neopharm Bulgaria Ltd (Sofia, Bulgaria). Before trial initiation, the investigator received 10 identical coded packs with trial interventions (5 packs with studied probiotic mix and 5 with placebo capsules) for 10 participants to be enrolled in the trial. Participants received the trial intervention from the investigators corresponding to the code provided by the randomization software on the day of enrollment. Each investigator had to assign 10 participants to guarantee that an equal number of participants was assigned to each treatment arm at each site. Everyone involved in the trial was blinded to the assignment until trial completion.

Trial Intervention

The studied probiotic mix contained 13 probiotic bacterial strains of 3 genera (*Lactobacillus* spp., *Bifidobacterium* spp., and *Bacillus coagulans*), 1 probiotic yeast strain (*S boulardii*), 3 prebiotics, and a vitamin B complex in an enterosolvent cellulose capsule with a total probiotic dose of 50×10^9 CFU/

capsule (see Supplementary Appendix Table 1 for the exact composition).

The studied probiotic mix was manufactured according to the local manufacturing guidelines and Good Manufacturing Practices for Dietary Supplements.

To ensure blinding, the placebo had the same appearance and composition as the active product, including the supplementary substances maltodextrin and magnesium stearate but without the live bacteria and yeast, prebiotic blend, and vitamin B complex.

The first dose of the studied probiotic mix/placebo was administered orally with the first dose of the antibiotic at the end of day 1 (visit 1). During the antibiotic treatment, 2 capsules of the studied probiotic mix (total probiotic dose of 100×10^9 CFU) or placebo were administered once per day, 2 hours before or after the antibiotic administration. After the completion of the antibiotic treatment, 1 capsule of the studied probiotic mix (total probiotic dose of 50×10^9 CFU) or placebo was administered once per day for 14 days.

Trial Outcomes

The primary outcome was the incidence of AAD,* defined as the number of participants who experienced at least 1 day of diarrhea compared to the total number of participants enrolled in the given trial intervention group. AAD was defined as ≥3 loose or liquid stools (types 5 to 7 according to Bristol Stool Form Scale [BSFS] [24]) over a period of 24 hours.

Secondary outcomes included:

1. Severity scale of AAD*: severe: ≥7 unformed/loose/liquid stools; moderate: 5 to 6 unformed/loose/liquid stools; mild: a change in the stool pattern −3 to 4 unformed/loose/liquid stools a day [25].
2. Duration of diarrhea*: the time until the first normalization of the stool form according to BSFS (presence of 1 or 2 sequential normal stools[ie, "soft and formed" or "hard and formed": types 1 to 4 per BSFS] [or lack of stool] for a period of 24 hours).
3. Antibiotic-associated symptoms*: ie, gastrointestinal complaints: nausea, abdominal pain, abdominal swelling/bloating, and gas formation/passing gas.
4. Quality of life measured by the visual analogue scale for the gastrointestinal quality of life (VAS-QoL) at visits 1 to 4. A lower score indicates a better QoL.
5. Investigator efficacy assessment (response to trial intervention) performed at visit 3, the efficacy of the trial intervention was classified as poor, good, or excellent.
6. Adverse events (AEs), including serious AEs.* For each AE, the severity and relationship to trial intervention were assessed by the investigator.

*The timeframe for these assessments was by 21 ± 2 days after the last antibiotic dose.

Data Management

At each site, data were collected by an investigator using an electronic Case Report Form. Each investigator's database was password-protected. Each participant received an electronic patient diary in which the participant daily reported the date, time, and received dose of trial intervention and the presence, frequency, and severity of observed parameters as described in the Trial outcomes section.

Sample Size and Statistical Analysis

The risk of AAD in the placebo group was assumed to be 19% [26]. To demonstrate a 35% reduction of risk for AAD (12% incidence rate) in the studied probiotic mix group with 80% power at a 5% significance level, 540 participants had to be included (270/group). In total, 630 participants were planned to be enrolled to allow a 15% dropout rate or lost to follow-up because unexpected complications unrelated to diarrhea.

As defined in the protocol, the analysis population for the efficacy assessments included participants without deviations in the written informed consent and having efficacy data at least from days 8 to 9 after the end of antibiotic treatment (participants who had received at least 75% of the total planned dose of the studied probiotic mix/placebo) (ie, a modified intention-to-treat population). Participants who discontinued early because of insufficient efficacy (including severe diarrhea requiring treatment) were not to be excluded from the efficacy assessment). The analysis population for the safety assessments included all participants who had given written informed consent and received at least 1 dose of trial intervention.

For comparison of continuous variables between groups, the Student *t*-test was used as appropriate. For comparison of dichotomic variables between groups, the Pearson χ^2 or standardized *Z*-test was used. Relative risk (RR) ratios and odds ratios (ORs) with a confidence interval (CI) of 95% were calculated for dichotomic results for the primary and secondary outcome analysis. All statistical tests were 2-sided with a significance level of 5%. The absolute risk reduction of AAD incidence and the number needed to treat to prevent the development of AAD were also calculated. Baseline characteristics and AEs were summarized descriptively.

RESULTS

Participant Disposition

In total, 575 participants were assessed for eligibility (Figure 1). Eleven of these were excluded based on the inclusion and exclusion criteria. Of 564 enrolled participants, 285 participants were randomly assigned to the studied probiotic mix group and 279 to the placebo group. Nine participants discontinued the trial early (3 on the studied probiotic mix and 6 on placebo), had no efficacy data, and were excluded from the efficacy analysis. The 555 remaining participants (282 on the studied

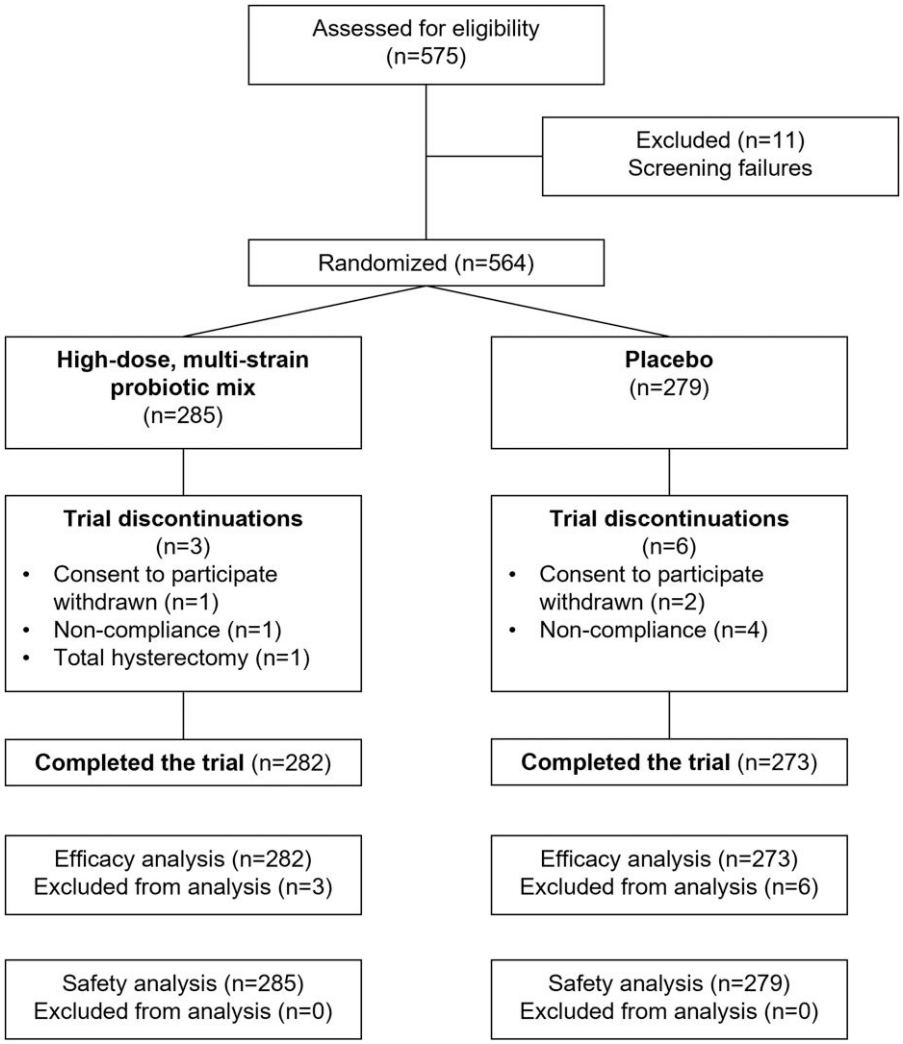


Figure 1. Flowchart of participant disposition. Screening failures included use of antibiotics within 60 d before screening, planned intake of antibiotics >10 d, and use of antidiarrheal medications. Although 1 AE of constipation in the studied probiotic mix group and 1 in the placebo group led to discontinuation of the trial intervention, these participants were not excluded from the efficacy analysis because they received more than 75% of the trial intervention. Nine participants discontinued the trial early (3 on the studied probiotic mix and 6 on placebo), had no efficacy data, and were excluded from the efficacy analysis. The clinical trial was conducted between 27 November 2022 and 25 April 2023. Abbreviation: AE, adverse event.

probiotic mix and 273 on placebo) completed the trial and were included in the efficacy analysis. Demographic data were comparable between the 2 trial intervention groups (Table 1). Most participants were female (62.3%) and the mean age was 40.8 ± 11.5 years. Most participants were diagnosed with bronchitis (38.0%), rhinosinusitis (11.9%), or otitis (9.5%) (see Supplementary Appendix Table 2). The frequency of prescription of the different antibiotic classes was as follows: cephalosporins (40.2%) as antibiotic treatment, followed by broad-spectrum penicillins (29.5%) and quinolones (28.5%) (Table 1). Tetracyclines were administered in a minority (1.8%) of the participants. The distribution of participants by antibiotic treatment was comparable between the 2 groups. See Supplementary Appendix Table 3 for a complete list of the antibiotics administered.

Primary Outcome: Incidence of AAD

Of the participants receiving the studied probiotic mix, 9.2% (26/282) developed AAD, whereas 25.3% (69/273) of the participants receiving placebo developed AAD (RR = 0.36 [95% CI, 0.24–0.55]; OR = 0.30 [95% CI, 0–0.79]; $P < .001$) (Figure 2). The absolute risk reduction of incidence of AAD was 16% in the studied probiotic mix group compared to the placebo group, and RR reduction was 64%. The number needed to treat to prevent the development of AAD was 6 (95% CI, 4.55–10.49). Regardless of the type of antibiotic administered, the incidence of AAD was consistently significantly lower in the studied probiotic mix group versus placebo group (Figure 2): 8.2% (7/85) versus 29.1% (23/79) for broad-spectrum penicillins ($P < .001$); 8.6% (8/93) versus 26.2% (17/65) for quinolones ($P = .003$); 11.3% (11/97) versus 23.0% (29/126) for cephalosporins ($P = .018$).

Table 1. Baseline Characteristics of Trial Participants

Characteristics	Probiotic Mix n = 282	Placebo n = 273	Total n = 555
Age (y), mean ± SD	40.9 ± 11.6	40.8 ± 11.4	40.8 ± 11.5
Sex (male), n (%)	106 (37.6)	103 (37.7)	209 (37.7)
Body height (cm), mean ± SD	169.82 ± 8.79	170.57 ± 9.52	170.19 ± 9.15
Body weight (kg), mean ± SD	71.26 ± 14.23	71.52 ± 14.22	71.39 ± 14.31
BMI (kg/m²), mean ± SD	23.33 ± 4.58	23.40 ± 4.68	23.36 ± 4.63
Comorbidities			
Hypertension, n (%)	36 (12.8)	32 (11.7)	68 (12.3)
Asthma, n (%)	13 (4.6)	14 (5.1)	27 (4.9)
COPD, n (%)	6 (2.1)	5 (1.8)	11 (2.0)
Diabetes, n (%)	3 (1.1)	7 (2.6)	10 (1.8)
Duration of antibiotic treatment (d), mean ± SD	7.23 ± 1.70	7.23 ± 1.77	7.23 ± 1.74
Antibiotic treatment			
Broad-spectrum penicillins, n (%)	85 (30.1)	79 (28.9)	164 (29.5)
Cephalosporins, n (%)	97 (34.4)	126 (46.2)	223 (40.2)
Quinolones, n (%)	93 (33.0)	65 (23.8)	158 (28.5)
Tetracyclines, n (%)	7 (2.5)	3 (1.1)	10 (1.8)

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; SD, standard deviation.

All trial participants who completed the trial were included.

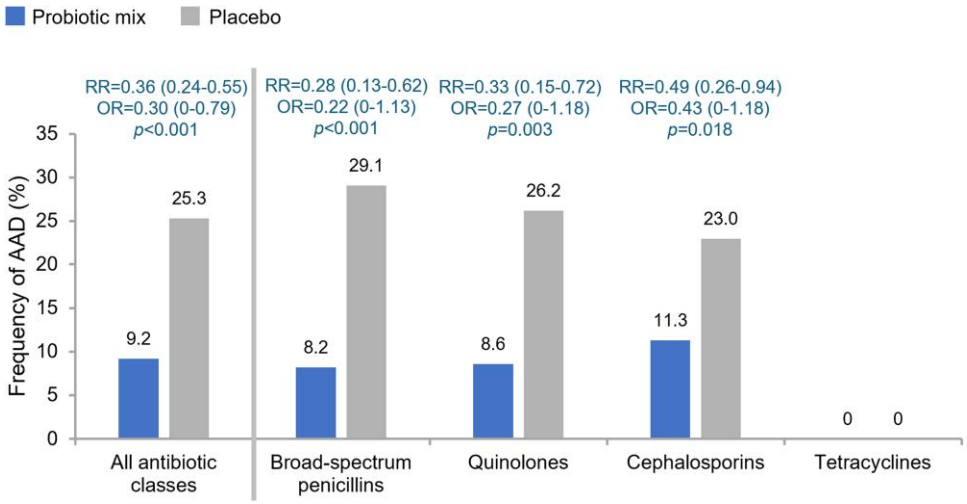


Figure 2. Frequency of participants with AAD (primary outcome) overall and by antibiotic treatment. Total n = 555 (probiotic mix/placebo: 282/273). RRs and ORs are accompanied by corresponding 95% CIs. *P*-values were calculated using the Pearson χ^2 test (2-sided). Abbreviations: AAD, antibiotic-associated diarrhea; CI, confidence interval; OR, odds ratio; RR, relative risk.

Because of the very low frequency of participants on tetracyclines, it was not possible to make any conclusion for this antibiotic treatment group.

Secondary Outcomes

Severity of AAD. The studied probiotic mix significantly decreased the severity of AAD (Figure 3*A*). Moderate AAD was reported less frequently in the studied probiotic mix group (1.1%; 3/282) compared to the placebo group (8.4%; 23/273) ($P < .001$). The same result was demonstrated for mild AAD (8.2% [23/282] vs 16.8% [46/273]) ($P = .002$). No severe AAD was reported in either group.

Duration of AAD. The mean (± standard deviation) duration of AAD was significantly lower in the studied probiotic mix group (2.6 ± 2.2 days) compared to the placebo group (3.7 ± 2.4 days) (mean difference: −1.12 days [95% CI, −2.18–0.05 days]; $P = .04$). In participants that experienced AAD, the duration of AAD was ≤2 days for the majority of participants receiving the studied probiotic mix (69.2%; 18/26) compared to 33.3%

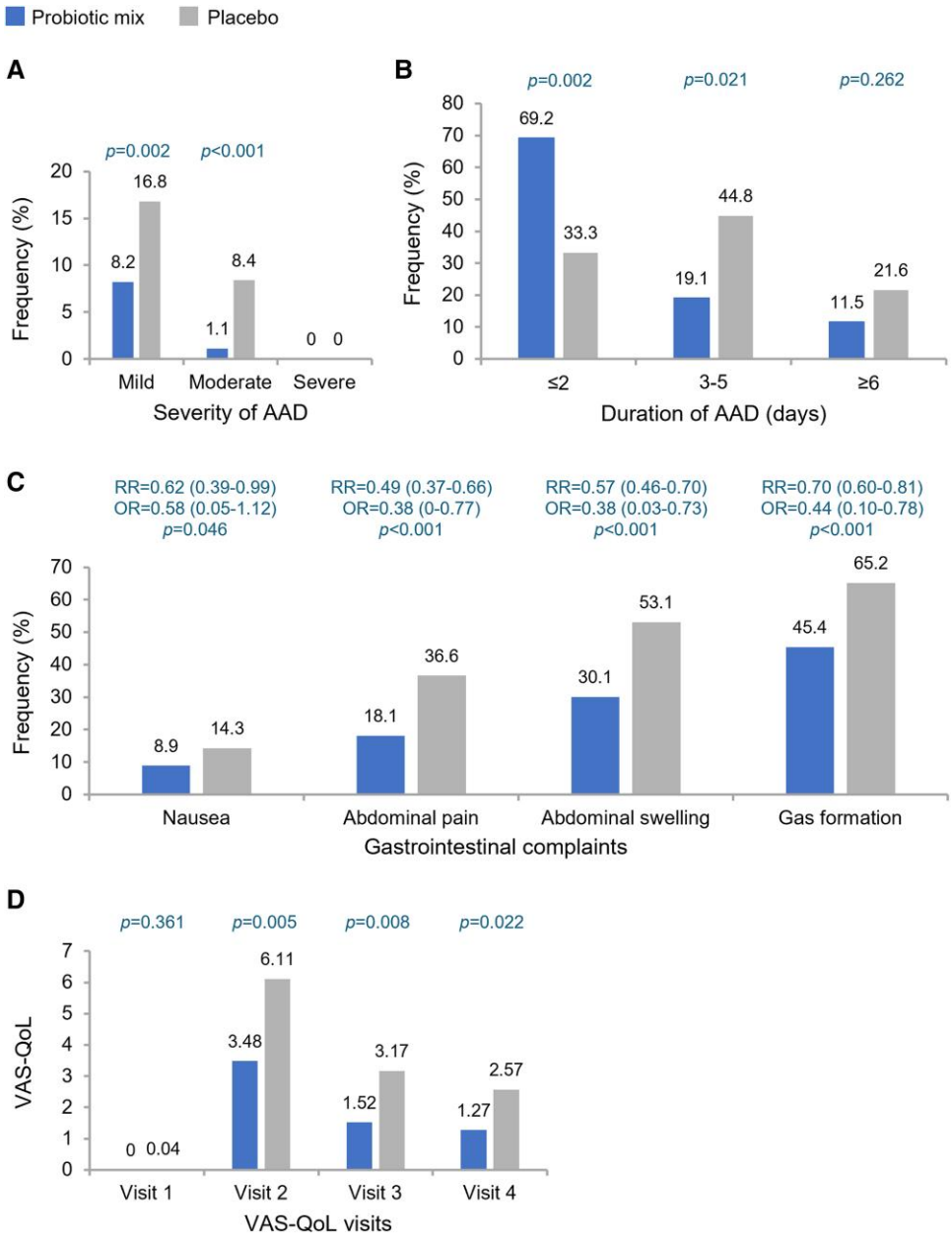


Figure 3. Secondary outcomes by trial intervention group. *A*, Severity of AAD: frequency of participants with mild, moderate, and severe AAD. Participants without AAD are not shown on the graph. *P*-values were calculated using the *Z*-test (2-sided). *B*, Duration of AAD in days: frequency of participants with an AAD duration of ≤2, 3–5, or ≥6 d. *P*-values were calculated using the *Z*-test (2-sided). *C*, Gastrointestinal complaints: frequency of participants with nausea, abdominal pain, abdominal swelling, and gas formation. *P*-values were calculated using the Pearson χ^2 test (2-sided). *D*, VAS-QoL per visit: visit 1 on day 1 and visits 2, 3, and 4 on 1 ± 2, 14 ± 2, and 21 ± 2 d after completion of antibiotic treatment, respectively. A lower VAS-QoL score indicates a better gastrointestinal QoL. *P*-values were calculated using the Student *t*-test (2–sided). Total n = 555 (probiotic mix/placebo: 282/273), note that in (*B*) only participants with AAD were included (n = 95 [probiotic mix/placebo: 26/69]). RRs and ORs are accompanied by corresponding 95% CIs. Abbreviations: AAD, antibiotic-associated diarrhea; CI, confidence interval; OR, odds ratio; QoL, quality of life; RR, relative risk; VAS–QoL, visual-analogue scale for the gastrointestinal quality of life.

(23/69) of participants receiving placebo ($P = .002$) (Figure 3*B*). The maximum duration of AAD was 10 days in the studied probiotic mix group (1 participant) and 9 days in the placebo group (2 participants) (see Supplementary Appendix Figure 1).

Gastrointestinal Complaints. The number of gastrointestinal complaints was significantly lower in the studied probiotic mix group compared to the placebo group (Figure 3*C*). For all gastrointestinal complaints, the RR reduction of occurrence was ≥30% in the studied probiotic mix group compared to the placebo group.

VAS-QoL. A lower VAS-QoL score indicates a better gastrointestinal QoL. During each scheduled visit, except for visit 1,

participants in the studied probiotic mix group reported a significantly better gastrointestinal QoL compared to participants in the placebo group (Figure 3*D*). Considering the entire trial period, the average gastrointestinal QoL was significantly better in the studied probiotic mix group (VAS-QoL score = 1.57) compared to the placebo group (VAS–QoL score = 2.97) ($P = .002$).

Investigator Efficacy Assessment—Response to Trial Intervention. As assessed by the investigator, most participants in the studied probiotic mix group (91.8%; 259/282) showed an excellent response to trial intervention compared to 59.3% (162/273) participants in the placebo group ($P < .001$) (see Supplementary Appendix Table 4). Poor response to the trial intervention was noted in only 1.8% (5/282) of participants receiving the studied probiotic mix compared to 4.4% (12/273) of participants receiving placebo ($P = .08$), although significance could not be reached, because of low incidence in both groups.

Adverse Events. Of 564 participants included in the safety analysis, only 1.8% (10/564) experienced an AE: 2.5% (7/285) in the studied probiotic mix and 1.1% (3/279) in the placebo group. All AEs per reported term occurred in at most 1 participant per group, except for the AE of lemon-yellow urine occurring in 1.8% (5/285) of participants receiving the studied probiotic mix and in none of the participants receiving placebo. The lemon-yellow urine is a known effect of excreted B vitamins, which were present in the studied probiotic mix but not in the placebo formulation. All AEs were mild (1.1%; 6/564) or moderate (0.7%; 4/564) in severity according to the investigator's assessment. None of the AEs was considered to be at least possibly related to the trial intervention by the investigator. One participant in the studied probiotic mix group (0.4%; 1/285) experienced a serious AE of total hysterectomy for which hospitalization was required. This led to discontinuation of the participant from the trial intervention and trial. Additionally, 1 AE of constipation in the studied probiotic mix group and 1 in the placebo group led to discontinuation of the trial intervention. A summary of the AEs per group can be found in Table 2 and a list of all AEs and related information can be found in Supplementary Appendix Table 5.

Table 2. Summary of the Adverse Events (Secondary Outcome)

	Probiotic Mix n = 285	Placebo n = 279	Total n = 564
Participants with at least 1 AE[a]	7 (2.5)	3 (1.1)	10 (1.8)
AE severity			
Mild AE	5 (1.8)	1 (0.4)	6 (1.1)
Moderate AE	2 (0.7)	2 (0.7)	4 (0.7)
Severe AE	0 (0.0)	0 (0.0)	0 (0.0)
AE considered related to trial intervention[b]	0 (0.0)	0 (0.0)	0 (0.0)
AE leading to discontinuation of trial intervention	2 (0.7)	1 (0.4)	3 (0.5)
AE leading to trial discontinuation	1 (0.4)	0 (0.0)	1 (0.2)
Serious AE	1 (0.4)	0 (0.0)	1 (0.2)
AE by reported term			
Lemon-yellow urine	5 (1.8)	0 (0.0)	5 (0.9)
Constipation	1 (0.4)	1 (0.4)	2 (0.4)
Headache	0 (0.0)	1 (0.4)	1 (0.2)
Vaginal candidiasis	0 (0.0)	1 (0.4)	1 (0.2)
Total hysterectomy	1 (0.4)	0 (0.0)	1 (0.2)

Abbreviation: AE, adverse event.

All values are n (%). All participants who had given written informed consent and received at least 1 dose of trial intervention were included.

[a]All participants with AEs only experienced 1 AE.

[b]Relationship to trial intervention was assessed by the investigator to be not, unlikely, possibly, probably, or definitely related. All AEs were considered not related or unlikely related to trial intervention by the investigator.

DISCUSSION

Prudent use of antibiotics in human medicine has been encouraged in recent years as a measure to combat antimicrobial resistance, which is 1 of the top 10 global public health threats [27]. Nevertheless, antibiotics are expected to remain fundamental for the treatment of bacterial infections and over- and misuse of antibiotics is still a common practice. As a result, many people are subjected to the different side effects of antibiotics, including AAD [28]. Therefore, a highly effective probiotic, especially developed for patients on antibiotic treatment is urgently needed.

The composition of the high-dose, multistrain probiotic mix (Sinquanon) was rationally designed to meet the needs of patients receiving antibiotic treatment to prevent AAD. The main advantage of the tested probiotic is that it includes 4 selected genera of probiotic strains with a total of 13 bacterial strains (7 *Lactobacillus* spp., 5 *Bifidobacterium* spp., *Bacillus coagulans*) and 1 yeast strain (*S boulardii*) at a high dose (50×10^9 CFU/capsule), in combination with a prebiotic blend and a vitamin B complex to support the probiotic strains. In the current trial, its efficacy and safety were evaluated for AAD prevention in adults to demonstrate the benefit of administering this probiotic mix as a routine add-on to antibiotic treatment in the outpatient setting.

Given the high frequency of antibiotic prescriptions at both pulmonology, as well as ENT practices, such sites were included to recruit participants in the current trial. In the outpatient setting, side effects of antibiotic treatment, such as AAD, often remain invisible to the attending physician, possibly leading to complications and thereby increasing overall morbidity and healthcare costs. Specific probiotics may reduce these additional complications.

The inclusion criterium specifying the acceptable antibiotic treatments was based on the frequency with which specific antibiotics are prescribed in pulmonology and ENT practices. Despite the important clinical role of macrolide antibiotics in

pulmonology, antibacterial regimens based on these agents were not encompassed in the inclusion criteria of this trial. Macrolides (eg, erythromycin, clarithromycin, roxithromycin, azithromycin) have been well established as potent motilin receptor agonists at clinically relevant concentrations; this pharmacological feature has been associated with intrinsic prokinetic activity in preclinical studies and documented gastrointestinal side effects, such as nausea, emesis, diarrhea, and abdominal pain [29–31]. Therefore, any occurrence of AAD during or after macrolide-based treatment would have been at least partly mediated by the aforementioned direct pharmacological mechanism, which makes its potential mitigation by a probiotic-based intervention implausible and beyond the primary scientific rationale of the trial.

In this trial, administration of the studied probiotic mix significantly reduced the RR of incidence of AAD compared to placebo by 64%. Although the numbers cannot be compared directly, the studied probiotic mix provided a higher reduction in the RR of incidence of AAD compared to meta-analysis findings for other probiotics tested for the prevention of AAD in adults (64% vs 37% [32] and 38% [33]). This is assumed to be due to the high dose of probiotic strains, the genera and strain diversity of the selected probiotic strains, and the inclusion of the prebiotic blend and vitamin B complex in the studied probiotic mix, which was specially designed for effective and safe prevention of AAD in adults on broad-spectrum antibiotic treatment. The overall incidence of AAD was within the typical ranges as reported in previous studies [3]. Although it should be noted that some cases of AAD may have been missed, because AAD may occur up to 8 weeks after antibiotic treatment [3] and in this trial, participants were followed for only 3 weeks after antibiotic treatment.

Next to a reduction in the incidence of AAD, in participants that experienced AAD, the trial data also showed a significant reduction in the duration of AAD in the studied probiotic mix group compared to the placebo group with a mean difference of approximately 1 day. These results indicate that patients who were impeded in their daily activities by AAD would be able to reassume their daily activities 1 day earlier when taking the studied probiotic mix. In light of the approximate 3-week treatment period (19–24 days) with the studied probiotic mix, the 1-day reduction in the duration of AAD can appear a modest benefit. However, this broad treatment period is relevant because antibiotic treatment takes 5 to 10 days, AAD can occur during or after antibiotic treatment, and the maximum duration of AAD in this trial was 10 days. Notably, this 1-day reduction in the duration of AAD is hypothesized to also lower the chance for severe AAD, including the colonization of opportunistic pathogens such as *C difficile*, although this should be further investigated.

Administration of the studied probiotic mix did not only significantly improve all included clinical outcomes, but also the gastrointestinal QoL of the participants. This trial is one of the few of its kind including this outcome measure, although as indicated by Goodman et al [32], such outcome measures are of importance for an accurate assessment of the cost-benefit of the studied therapy. One randomized trial, which found no evidence for effectiveness of a high-dose blend of lactobacilli and bifidobacteria in the prevention of AAD in older people admitted to the hospital, reported a similar QoL in the probiotic and placebo group [34]. On the contrary, in the current trial, the response to trial intervention was significantly better in the studied probiotic mix group compared to the placebo group and was assessed by the investigators as an excellent response for most participants in the studied probiotic mix group.

The few reported AEs were mild or moderate in severity and none was considered to be at least possibly related to the trial intervention. Moreover, there were no clinically relevant differences in the incidence of AEs between the studied probiotic mix and the placebo groups, demonstrating that the studied probiotic mix is safe and well-tolerated in adults.

A limitation of the trial is that no tests for *C difficile* or microbiome evaluations were performed as this was not standard clinical practice at the site. Future studies may investigate shifts in the microbiome before, during, and after intake of the probiotic mix. Another limitation entails that the use of analgesics containing codeine (which are known to prevent diarrhea) was not included in the exclusion criteria.

Future trials should be conducted to verify the efficacy and safety of the studied probiotic mix in the hospital setting and in children.

CONCLUSIONS

Administration of the studied probiotic mix significantly reduced the incidence of AAD compared to placebo in adults on broad spectrum antibiotic treatment.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. All authors have delivered scientific lectures on a given problem for various pharmaceutical companies, including Neopharm Bulgaria.

Patient consent statement. Each patient's written consent was obtained and the design of the work has been approved by an independent local ethical committee (Research Ethics Committee at Medical University—Sofia [Number 15/18.11.2022]).

Data availability. The data that support the findings of this trial are available from the corresponding author upon reasonable request.

Author Contributions. Conceptualization of the trial: V.H., N.S., G.M.; trial methodology: V.Y., R.N.; investigation of participants: M.E., R.B.; supervision of the trial: K.D., B.B.; validation of trial data: N.S., S.T.; project administration: B.B., V.Y.; visualization of trial data: K.D., M.E.; resources: R.N., V.H.; data curation, formal analysis, software: V.H. All authors contributed to data analysis, drafting, and revising the manuscript. All authors reviewed and approved the final manuscript.

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