Influence of a probiotic mixture on antibiotic induced microbiota disturbances

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Abstract

AIM: To study the effect of probiotic consumption on the faecal microbiota during and after antibiotic exposure.

METHODS: A randomized, double-blind, placebo-controlled, parallel group study with a two species probiotic combination [Lactobacillus acidophilus (L. acidophilus) ATCC 700396 and Bifidobacterium lactis (B. lactis) ATCC SD5220] on healthy adults during and after antibiotic treatment (amoxicillin 875 and 125 mg clavulanate). The dominant faecal microbiota was studied by real time-polymerase chain reaction to determine if this probiotic preparation could facilitate restoring the microbiota to its pre-antibiotic state and influence the prevalence of beta-lactam resistance. Gastrointestinal symptoms were recorded by questionnaire and Bristol stool scale.

RESULTS: Subjects on the probiotic combination had significantly higher faecal counts of L. acidophilus ATCC 700396 and B. lactis at day 8 (end of antibiotic treatment period) vs those on placebo. Furthermore, subjects on the probiotic combination had significantly higher faecal counts of L. acidophilus ATCC 700396 and B. lactis at Day 15 (end of probiotic treatment) vs those on placebo. Lactobacillus counts remained stable in the probiotic group over the course of the study, while Clostridium XIV group was higher at the end of the study and closer to baseline levels; this in contrast to the placebo group. Beta-lactam resistance increased after antibiotic exposure and was not different between both treatment groups. Gastrointestinal symptoms were generally mild and did not differ between the treatment groups, which correlates with the generally small changes in the microbiota.

CONCLUSION: Consumption of the probiotic combination mainly leads to an increase in the faecal levels of the species included in the preparation.

Key words: Probiotic; Antibiotic treatment; Amoxicillin/clavulanate; Microbiota; Beta-lactamases; Lactobacillus acidophilus; Bifidobacterium lactis

Core tip: The influence of a probiotic combination on the stability of the intestinal microbiota was studied using molecular techniques. Most published studies have relied on culturing or have only looked at symptomology. Furthermore, this was studied in an antibiotic challenge setting to limit variability.

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INTRODUCTION

Although the antimicrobial properties of antibiotics have provided great medical benefits, they may also affect the composition and activity of, in particular, the intestinal microbiota. This disturbance in the balance and diversity of the composition of the normal intestinal microbiota has been identified as the major factor involved in the pathogenesis of antibiotic associated diarrhoea (AAD)\(^9\). The magnitude of these changes is influenced by the dose, type and duration of antibiotic use, along with the capability of the intestinal microbiota to resist colonization changes.

Treatment possibilities for AAD are limited, but probiotics have been suggested as a potential way to counteract the potential negative effects of antibiotics. The Food and Agricultural Organization of the United Nations and World Health Organization have defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”\(^6\). Various strains of probiotics have been shown to protect against bacterial and viral enteropathogens by producing inhibitory antimicrobial substances such as organic acids, hydrogen peroxide and bacteriocins, and demonstrating competitive inhibition for bacterial adhesion sites on intestinal epithelial surfaces\(^7\). Such properties may make probiotics good candidates for stabilizing the intestinal microbiota during antibiotic challenge. Selected probiotic preparations have been shown to reduce antibiotic induced microbiota disturbances\(^8\). Furthermore, many strains of probiotics have also been found to reduce the incidence of AAD; a recent meta-analysis concluded that probiotics are associated with a reduced risk for AAD\(^9\).

The primary objective of the present study was to investigate the effect of a specific combination of probiotic strains on the incidence of antibiotic induced microbiota disturbances. The secondary objectives were to investigate the influence of probiotics on quality of life and stool consistency during and following antibiotic use. The study was triple-blind, randomized, placebo controlled, and stratified by gender at a ratio of 1:1. After successful screening, all volunteers received amoxicillin and clavulanate daily from day 1 to 7 and were randomly allocated to receive either probiotic or placebo daily from day 1 to 14, where after the volunteers had a 7 d follow up period.

MATERIALS AND METHODS

The study was reviewed and approved by the Therapeutic Products Directorate, in consultation with the Natural Health Products Directorate, Health Canada, and Institutional Review Board Services (Aurora, ON, Canada), and conducted in accordance with the Declaration of Helsinki.

Study design

The study was triple-blind, randomized, placebo controlled with two parallel study groups. Participants were stratified by gender at a ratio of 1:1. After successful screening, all volunteers received amoxicillin and clavulanate daily from day 1 to 7 and were randomly allocated to receive either probiotic or placebo daily from day 1 to 14, where after the volunteers had a 7 d follow up period.

For the study, 111 participants were screened; 80 were enrolled (Figure 1). The inclusion criteria were male or female aged 18 to 50 years; if female, either not of child bearing potential or using a medically approved method of birth control; body mass index 18.0: 29.9 kg/m\(^2\); healthy as determined by laboratory results, medical history and physical exam; agreed not to change current dietary habits (with the exception of avoiding pro- and prebiotics) and activity/training levels during the course of the study; gave voluntary, written, informed consent to participate in the study. Exclusion criteria were - women who were pregnant, breastfeeding, or planning to become pregnant during the course of the trial; body mass index $\geq$ 30 kg/m\(^2\); average number of formed bowel movements $> 3$ per day or $< 3$ per week; smokers (ex-smokers must have quit at least 3 mo prior); participation in a clinical research trial within 30 d prior to randomization; use of antibiotics within 60 d prior to randomization; habitual use of pro- and/or prebiotic products; followed a vegetarian or vegan diet; unstable medical conditions; history of chronic gastrointestinal disorders; alcohol use $> 2$ standard alcoholic drinks per day and/or alcohol or drug abuse within past year; allergy or sensitivity to test product ingredients or antibiotic (amoxicillin and clavulanate), allergy to any penicillin antibiotic or cephalosporin antibi-otic; individuals who were cognitively impaired and/or unable to give informed consent; any other condition which, in the investigator’s opinion, may adversely affect the subject’s ability to complete the study or its measures or which may pose significant risk to the subject.

Study products

The study products consisted of $12.5 \times 10^9$ CFU/d Lac-tobacillus acidophilus (L. acidophilus) ATCC 700396 and $12.5 \times 10^9$ CFU/d Bifidobacterium animalis (B. animalis) ssp. lactis ATCC SD5220 (Danisco USA, Madison, WI, United States) in a hypromellose capsule. Maltodextrin was used as an excipient. The placebo consisted of the same capsule with only maltodextrin. At the end of the study, viable counts were determined and found to have deviated less than 10% from the target count.

The antibiotic used was Augmentin (Apotex, Toronto, Canada); 875 mg amoxicillin and 125 mg clavulanate.

Compliance

Compliance was assessed by counting the returned study product and antibiotic at each visit. Compliance was calculated as a percentage by determining the number of dosage units consumed divided by the number expected to have been taken multiplied by 100%. In the event of a discrepancy between the information in the subject diary and the amount of study product returned, calculations were based on the product returned unless an explanation for loss of product was provided. Participants found to have a compliance of $< 80\%$ or $> 120\%$ at any visit were counselled. A compliance of $< 70\%$ or $> 130\%$ was considered as non-compliant and any subject demonstrating non-compliance for two consecutive visits was to be withdrawn from the study. Compliance rates over 100% were explained by a visit later then intended and
additional consumption of study product.

**Outcomes**
The primary objective was to evaluate the maintenance of the intestinal microbiota composition during antibiotic (amoxicillin and clavulanate) treatment by quantitative real-time polymerase chain reaction (qPCR). To this end, the following commensal and potential pathogenic microbial groups were analysed from the faecal samples: *Lactobacillus* spp.[8], *L. acidophilus* ATCC 700396[7], *Bifidobacterium*[8], *B. lactis*[9,10], *Bacteroides*[11], *C. difficile*[6], *Clostridium cluster XIV*[12] and *Enterobacteriaceae*[13] by qPCR; using a ABI 7500 FAST sequencing detection system (Applied Biosystems Foster City, United States). Ten-fold dilution series (10 pg and 1 ng) of DNA from the standard strains were used for the standard curves. For the determination of DNA, triplicates of each sample were run, and the mean quantity per gram faecal wet weight was calculated. The total bacterial count was analyzed by flow cytometry as described previously[14].

Prevalence of antibiotic resistance caused by the extended-spectrum beta-lactamases (ESBL) was analyzed by a PCR and hybridisation combined method using a commercial Multiplex ESBL kit (BIORON Diagnostics).
GmbH, Ludwigshafen, Germany). This kit detects a selection of potentially ESBL-positive bacteria by detecting all variants of the genes bla\textsubscript{TEM}, bla\textsubscript{SHV}, bla\textsubscript{CTX-M} and relevant ESBL phenotypic variants of bla\textsubscript{Bla\textsubscript{LAM}.}

Secondary outcomes consisted of Gastrointestinal Symptom Rating Scale (GSRS), bowel habit scores; frequency and consistency (Bristol Stool scale) and adverse events. The GSRS is a disease-specific questionnaire of 15 items combined into five symptom clusters depicting reflux, abdominal pain, indigestion, diarrhoea and constipation; with a scale from 1 to 7 for no to serious symptoms. The GSRS is well-documented\cite{16} and norm values for a general population have been established\cite{17}. Bowel habits were scored on a diary; for number of bowel movements, straining to start defecation, straining to stop defecation, feeling of incomplete defecation and use of laxatives. Finally, stool score was formed according to the Bristol Stool scale which describes and depicts the form of the faeces on a 7 point scale, from hard (1) to watery (7)\cite{17}.

Adverse events, especially those for which the relationship to investigational product was suspected, were to be recorded and followed up on until they returned to baseline status or stabilized. In the rare event of microbial overgrowth with subject displaying the symptoms of a bacterial infection, the study physician was instructed to prescribe an antibiotic to which the strains were known to be susceptible.

Sample size
A per group sample size of 40 participants was required to detected a clinically significant difference of 10% at 80% power, \(a = 0.05\) (2-sided), 15% difference in statistical methods, allowing for a 20% attrition rate\cite{18}.

Randomization
A randomization schedule was created by the manufacturer. Participants were stratified by gender. A total of 112 randomizations were provided (56 males and 56 females), to account for additional recruitment, lost bottles, etc. The unique randomization numbers (112) were created using randomizer.org for each test product and divided over 28 blocks of 4.

Statistical analysis
Data was analyzed on the basis of intention-to-treat. Frequency counts and proportions were used to describe categorical variables. Subject demographics were compared between groups using unpaired Student \(t\) test, Fisher exact test or \( \chi ^ 2 \) test as appropriate. For outcomes with continuous variables, comparisons of changes over time were analyzed by paired Student \(t\) test of Wilcoxon signed-rank test. Differences between treatments were analyzed by unpaired \(t\) test or Mann-Whitney \(U\) test.

RESULTS

Study participants and demographics
Subject demographics and characteristics were similar for both treatment groups, with the exception of alcohol use \((P = 0.013)\), where the probiotic group tended to have more occasional drinkers (Table 1). Two participants in the placebo group and four participants in the probiotic group did not complete the study (Figure 1).

Compliance
Compliance of antibiotics use was greater than 99% (standard deviation 2.7%) in both treatment groups. Compliance of probiotic/placebo use was greater than 100% (standard deviation 5.5% for the first week and 9.4% for the second week).

Microbiota composition
At baseline, no differences were detected between the two groups for any of the tested microbial taxa (Table 2).

Subjects randomized to receive probiotics had increased faecal counts of \textit{L. acidophilus} ATCC 700396 at the end of antibiotic treatment period and at the end of study product treatment period compared to those receiving placebo (Table 2). When comparing between groups, the probiotic group had significantly higher levels of \textit{B. lactis} and \textit{L. acidophilus} ATCC 700396 than the placebo group as long as the study products were consumed. \textit{Lactobacillus} levels were not affected by the antibiotic in the probiotic group; this in contrast to the placebo group. Furthermore, the \textit{B. lactis} levels were restored to base line after completing probiotic consumption. In the placebo group, \textit{B. lactis} levels were still not restored to baseline at the end of the study (Table 2).

Within groups, after one week of antibiotic and probiotic or placebo consumption, total bacterial counts and \textit{Clostridium} cluster XIV counts both decreased from baseline. On the other hand, \textit{Enterobacteriaceae} were significantly increased in both groups \((P < 0.001)\). In the placebo group, \textit{Lactobacillus} spp. levels and \textit{B. lactis} levels were reduced compared to baseline, while in the probiotic group did not complete the study (Figure 1).
After follow up, which was the last week of the study (day 15) and after 1 wk follow-up period (day 22). Data are expressed as mean ± SD.

### Table 2  Bacterial counts

|                        | Probiotic | Placebo | **P value (within group)** | **P value** |
|------------------------|-----------|---------|---------------------------|-------------|
|                        |           |         | Within group | Between group |
| Total bacteria         |           |         |              |              |
| Baseline               | 10.89 ± 0.22 | - | 10.81 ± 0.23 | - | 0.067 |
| End of antibiotic + probiotic/placebo | 10.58 ± 0.43 | < 0.001 | 10.49 ± 0.38 | < 0.001 | 0.177 |
| End of probiotic/placebo | 10.75 ± 0.30 | 0.003 | 10.77 ± 0.26 | 0.399 | 0.735 |
| End of follow-up       | 10.87 ± 0.24 | 0.527 | 10.79 ± 0.31 | 0.568 | 0.221 |
| Lactobacillus          |           |         |              |              |
| Baseline               | 7.40 ± 0.79 | - | 7.42 ± 1.56 | - | 0.391 |
| End of antibiotic + probiotic/placebo | 7.13 ± 0.88 | 0.104 | 6.91 ± 1.45 | 0.032 | 0.642 |
| End of probiotic/placebo | 7.42 ± 0.77 | 0.944 | 7.07 ± 1.38 | 0.030 | 0.331 |
| End of follow-up       | 7.16 ± 1.50 | 0.375 | 6.96 ± 1.87 | 0.149 | 0.851 |
| *Lactobacillus acidophilus* ATCC 700396 |           |         |              |              |
| Baseline               | 1.27 ± 2.20 | - | 0.93 ± 1.73 | - | 0.446 |
| End of antibiotic + probiotic/placebo | 2.39 ± 2.98 | 0.052 | 1.21 ± 2.07 | 0.407 | 0.035 |
| End of probiotic/placebo | 2.15 ± 2.79 | 0.245 | 0.79 ± 1.54 | 0.933 | 0.011 |
| End of follow-up       | 1.60 ± 2.51 | 0.286 | 2.13 ± 2.74 | 0.021 | 0.498 |
| *Bifidobacterium*      |           |         |              |              |
| Baseline               | 8.62 ± 1.60 | - | 7.97 ± 1.99 | - | 0.087 |
| End of antibiotic + probiotic/placebo | 8.20 ± 1.08 | 0.016 | 7.83 ± 1.78 | 0.350 | 0.642 |
| End of probiotic/placebo | 8.72 ± 0.79 | 0.759 | 8.01 ± 2.13 | 0.805 | 0.142 |
| End of follow-up       | 8.52 ± 1.60 | 0.466 | 8.12 ± 1.57 | 0.422 | 0.236 |
| *Bifidobacterium lactis* |           |         |              |              |
| Baseline               | 8.81 ± 0.50 | - | 8.82 ± 0.69 | - | 0.914 |
| End of antibiotic + probiotic/placebo | 8.41 ± 1.48 | 0.054 | 8.20 ± 0.64 | < 0.001 | 0.008 |
| End of probiotic/placebo | 8.79 ± 0.63 | 0.904 | 8.42 ± 0.67 | < 0.001 | 0.013 |
| End of follow-up       | 8.67 ± 0.49 | 0.206 | 8.49 ± 0.74 | < 0.001 | 0.185 |
| *Bacteroides*          |           |         |              |              |
| Baseline               | 9.14 ± 0.56 | - | 8.97 ± 0.48 | - | 0.161 |
| End of antibiotic + probiotic/placebo | 9.06 ± 0.87 | 0.981 | 9.00 ± 0.61 | 0.629 | 0.345 |
| End of probiotic/placebo | 8.98 ± 0.69 | 0.050 | 8.85 ± 0.63 | 0.194 | 0.429 |
| End of follow-up       | 9.14 ± 0.63 | 0.972 | 8.91 ± 0.54 | 0.479 | 0.079 |
| *Enterobacteriaciae*   |           |         |              |              |
| Baseline               | 6.92 ± 0.83 | - | 6.74 ± 1.33 | - | 0.734 |
| End of antibiotic + probiotic/placebo | 7.80 ± 1.16 | < 0.001 | 7.68 ± 1.07 | < 0.001 | 0.531 |
| End of probiotic/placebo | 6.88 ± 0.73 | 0.944 | 6.87 ± 0.72 | 0.732 | 0.947 |
| End of follow-up       | 6.89 ± 0.55 | 0.956 | 6.75 ± 1.33 | 0.553 | 0.770 |
| *Clostridium difficile*|           |         |              |              |
| Baseline               | 2.85 ± 1.44 | - | 2.90 ± 1.29 | - | 0.563 |
| End of antibiotic + probiotic/placebo | 3.42 ± 2.30 | 0.283 | 2.95 ± 2.54 | 0.632 | 0.281 |
| End of probiotic/placebo | 3.13 ± 1.42 | 0.566 | 2.56 ± 1.76 | 0.126 | 0.077 |
| End of follow-up       | 3.08 ± 1.44 | 0.712 | 2.91 ± 1.68 | 0.475 | 0.935 |
| *Clostridium group XIV*|           |         |              |              |
| Baseline               | 10.04 ± 0.24 | - | 9.92 ± 0.39 | - | 0.073 |
| End of antibiotic + probiotic/placebo | 9.36 ± 0.72 | < 0.001 | 9.36 ± 0.45 | < 0.001 | 0.582 |
| End of probiotic/placebo | 9.85 ± 0.32 | < 0.001 | 9.74 ± 0.37 | 0.004 | 0.146 |
| End of follow-up       | 9.94 ± 0.25 | 0.046 | 9.75 ± 0.36 | 0.006 | 0.011 |

Bacterial counts (log10 counts/g wet weight) at baseline (day 1), after 1 wk treatment period with antibiotic + probiotic or placebo (day 8), after 1 wk of supplementation with probiotic or placebo only (day 15) and after 1 wk follow-up period (day 22). Data are expressed as mean ± SD.

After the additional week on probiotic or placebo, without antibiotics, *Clostridium* cluster XIV levels remained significantly reduced in both groups when compared to baseline. In the placebo group, *Lactobacillus* levels and *B. lactis* levels remained below baseline. In the probiotic group, *Lactobacillus* levels and *B. lactis* levels were restored to base-line but total bacterial numbers remained and *Bacteroides* remained below baseline (Table 2).

After follow up, which was the last week of the study where volunteers did not receive either probiotic or placebo, *Clostridium* cluster XIV levels remained reduced in both groups when compared to baseline. In the placebo group, levels of *L. acidophilus* ATCC 700396 increased to above baseline levels while *B. lactis* remained below baseline (Table 2).

**Prevalence of antibiotic resistance**

One subject within each group had a positive baseline sample for beta-lactam resistance. After antibiotic treatment, 16 participants in the probiotic group and 14 participants in the placebo group showed a positive signal for beta-lactam resistance ($P = 0.924$). None of the samples were positive for ESBL production.
### Table 3 Gastrointestinal Symptom Rating Scale scores for the study participants

|                          | Probiotic  | P value | Placebo   | P value |
|--------------------------|------------|---------|-----------|---------|
|                          | (n = 40)   | Within group | (n = 40) | Within group |
| Stomach ache or pain     | Baseline   | 1.10 ± 0.38 | -        | 1.32 ± 0.73 | -        | 0.101 |
| End of antibiotic        | 1.82 ± 1.32 | 0.0021    | 1.80 ± 1.26 | 0.0323    | 0.991 |
| End of treatment         | 1.48 ± 1.22 | 0.1031    | 1.45 ± 1.01 | 0.6323    | 0.8293 |
| End of study             | 1.38 ± 0.98 | 0.1311    | 1.48 ± 1.15 | 0.6711    | 0.7411 |
| Nausea                   | Baseline   | 1.00 ± 0.00 | -        | 1.23 ± 0.66 | -        | 0.0223 |
| End of antibiotic        | 1.50 ± 1.26 | 0.0221    | 1.60 ± 1.15 | 0.0883    | 0.3603 |
| End of treatment         | 1.27 ± 0.93 | 0.0541    | 1.15 ± 0.36 | 0.6083    | 0.8283 |
| End of study             | 1.12 ± 0.79 | > 0.9999  | 1.15 ± 0.53 | 0.6083    | 0.1873 |
| Rumbling in stomach      | Baseline   | 1.55 ± 0.75 | -        | 1.50 ± 0.75 | -        | 0.7433 |
| End of antibiotic        | 1.95 ± 1.34 | 0.0381    | 1.80 ± 1.36 | 0.1873    | 0.2343 |
| End of treatment         | 1.73 ± 1.26 | 0.5651    | 1.42 ± 0.87 | 0.4883    | 0.2783 |
| End of study             | 1.50 ± 0.93 | 0.3881    | 1.35 ± 0.77 | 0.1703    | 0.2673 |
| Bloating                 | Baseline   | 1.25 ± 0.44 | -        | 1.30 ± 0.76 | -        | 0.5473 |
| End of antibiotic        | 1.85 ± 1.48 | 0.0101    | 1.32 ± 0.76 | 0.9363    | 0.0713 |
| End of treatment         | 1.50 ± 0.96 | 0.1921    | 1.32 ± 0.80 | > 0.9999  | 0.2423 |
| End of study             | 1.50 ± 0.88 | 0.0961    | 1.40 ± 1.08 | 0.7193    | 0.2963 |
| Flatulence               | Baseline   | 1.57 ± 0.81 | -        | 1.52 ± 0.78 | -        | 0.8343 |
| End of antibiotic        | 2.00 ± 1.36 | 0.0291    | 1.62 ± 0.98 | 0.8573    | 0.2393 |
| End of treatment         | 1.70 ± 1.30 | > 0.9999  | 1.45 ± 0.99 | 0.3313    | 0.3033 |
| End of study             | 1.52 ± 1.13 | 0.2911    | 1.45 ± 0.85 | 0.4923    | 0.6893 |
| Diarrhea                 | Baseline   | 1.18 ± 0.55 | -        | 1.20 ± 0.79 | -        | 0.5123 |
| End of antibiotic        | 1.92 ± 1.53 | 0.0031    | 1.73 ± 1.26 | 0.0503    | 0.6143 |
| End of treatment         | 1.45 ± 1.28 | 0.2601    | 1.20 ± 0.61 | > 0.9999  | 0.6703 |
| End of study             | 1.35 ± 1.05 | 0.3891    | 1.15 ± 0.70 | 0.8923    | 0.0943 |
| Loose stools             | Baseline   | 1.25 ± 0.63 | -        | 1.25 ± 0.71 | -        | 0.7903 |
| End of antibiotic        | 1.70 ± 1.07 | 0.0021    | 1.48 ± 0.75 | 0.1093    | 0.4923 |
| End of treatment         | 1.48 ± 0.99 | 0.2411    | 1.20 ± 0.46 | 0.7843    | 0.3223 |
| End of study             | 1.25 ± 0.44 | 0.8241    | 1.27 ± 0.75 | > 0.9999  | 0.5033 |
| Bowel movement           | Baseline   | 1.25 ± 0.67 | -        | 1.40 ± 0.84 | -        | 0.2913 |
| End of antibiotic        | 1.73 ± 1.20 | 0.0321    | 1.73 ± 1.06 | 0.1163    | 0.7393 |
| End of treatment         | 1.52 ± 1.13 | 0.2081    | 1.35 ± 0.77 | 0.8133    | 0.7123 |
| End of study             | 1.38 ± 1.10 | 0.7171    | 1.32 ± 0.86 | 0.5683    | 0.8163 |
| Acid reflux              | Baseline   | 1.55 ± 0.71 | -        | 1.55 ± 0.64 | -        | 0.8503 |
| End of antibiotic        | 1.60 ± 0.90 | 0.8051    | 1.55 ± 0.81 | 0.9603    | 0.8903 |
| End of treatment         | 1.52 ± 0.72 | 0.8941    | 1.35 ± 0.66 | 0.1293    | 0.2103 |
| End of study             | 1.27 ± 0.60 | 0.0341    | 1.35 ± 0.66 | 0.1623    | 0.4963 |
| Constipation             | Baseline   | 1.27 ± 0.51 | -        | 1.25 ± 0.59 | -        | 0.5173 |
| End of antibiotic        | 1.52 ± 1.15 | 0.2661    | 1.65 ± 1.25 | 0.0073    | 0.6253 |
| End of treatment         | 1.42 ± 1.11 | 0.8221    | 1.30 ± 0.76 | 0.8573    | 0.6053 |
| End of study             | 1.60 ± 1.24 | 0.1081    | 1.65 ± 1.25 | 0.0343    | 0.7083 |
| Overall GSRS             | Baseline   | 1.27 ± 0.280 | -        | 1.22 ± 0.319 | -        | 0.4443 |
| End of antibiotic        | 1.60 ± 0.76 | < 0.0011  | 1.54 ± 0.58 | 0.0073    | 0.9693 |
| End of treatment         | 1.45 ± 0.79 | 0.5091    | 1.28 ± 0.33 | 0.1923    | 0.4813 |
| End of study             | 1.36 ± 0.70 | 0.7401    | 1.32 ± 0.57 | 0.2643    | 0.8643 |

Data are expressed as mean ± SD. 1 After a P value indicates that it was obtained from a non-parametric test, such as the Wilcoxon or Mann-Whitney U test. This is done whenever the values being summarized are significantly non-normally distributed, as assessed by the Anderson-Darling test. GSRS: Gastrointestinal Symptom Rating Scale.

**GSRS**

In general, GSRS scores were low; 2 or less; i.e., no or slight discomfort. Nausea was reported more in the placebo group at baseline compared to the probiotic group (Table 3). No other differences were reported between groups at baseline.

Following antibiotic consumption, both groups reported increased stomach ache or pain, nausea and diarrhea; the numbers were similar between the groups and normalized in the following weeks (Table 3). Though not significantly different between the groups, the probiotic group reported a reduction in acid reflux after the follow-up week (Table 3). On the other hand, participants in the placebo group reported more constipation after the antibiotic and placebo week (P = 0.007) and after the follow-up week (P = 0.034).

Over all, total GSRS scores were different for both groups only after the week with antibiotics; probiotic (P < 0.001) and placebo (P = 0.007) group. There was no difference between groups for the overall GSRS score at any of the assessed time points.

**Bowel habits**

Although volunteers in the probiotic group had a sig-
significant increase in bowel movements after antibiotic administration \((P = 0.032)\) this was not different from the placebo group.

**Bristol stool scale**

Subjects in both groups reported increased Bristol stool scale values with the highest stool scale value on day three of the antibiotic period. The probiotic group tended to have somewhat looser stools than the placebo group.

**Adverse events**

A total of 59 adverse events were reported during the study by 35 participants. All adverse events resolved before the end of study. There was no significant difference in the number of participants reporting any adverse event between treatment groups; 16 in the probiotic group and 19 in the placebo group. In the probiotic group, one subject withdrew during the antibiotic supplementation period due to upset stomach (Figure 1). No serious adverse events were reported during the study.

**DISCUSSION**

Antibiotics have brought great benefits to medical practice. However, their antimicrobial activities affect not just the targeted pathogen, but also the endogenous microbiota of the host. This disturbance in microbiota composition and activity is considered to be one of the reasons for AAD\cite{1,19}. Most studies on AAD and probiotics use patients as their study population. However, the use of patients introduces variability as the participants have different underlying diseases and usually get prescribed various antibiotics for various lengths of time and at different doses. When studying the effect of antibiotics on the intestinal microbiota and how probiotics may influence this, patients are not usually able to provide a baseline sample. The design of the current study, using healthy volunteers that took the same antibiotic for the same length of time, allowed the baseline to be established and eliminated variation that may have resulted from differing lengths and doses of antibiotic usage. The study design does, however, not allow for conclusions on other antibiotic regimens and/or probiotic preparations. A similar study set up indicated that a combination of five probiotic strains was able to maintain the overall intestinal microbiota composition\cite{8}. However, the study did not investigate specific microbial groups and the consumed probiotic strains by molecular methods, as was done in the present study.

The antibiotic induced limited changes in the faecal microbiota. The changes that were observed, were small and although statistically significant, the biological relevance may be limited. Total bacterial numbers (by faecal wet weight) were reduced in both treatment groups, which can be explained by the looser stools that were produced. The reduction in lactobacilli in the placebo group was not observed in the probiotic group and may be explained by the consumption of the probiotic that contained a *Lactobacillus* and may suggest a stabilisation of the faecal *Lactobacillus* levels by the probiotic. Likewise, levels of *L. acidophilus* ATCC 700396 and *B. lactis* were higher or more stable in the probiotic group; which was also likely related to the consumption of these strains/species. The apparent increase in *L. acidophilus* ATCC 700396 levels in the placebo group at the end of the follow up period can be explained by the inadvertent consumption of probiotic products by some volunteers. *Enterobacteriaceae* were increased in both groups after the antibiotic consumption and this was not influenced by the consumption of probiotics. *C. difficile* was not influenced by either the antibiotic or the probiotic, which was contrary to earlier observations where *L. acidophilus* ATCC 700396, together with *L. rhamnosus* HN001 was able to reduce the level and number of participants carrying *C. difficile*\cite{6}.

Only broad-spectrum beta-lactamases could be detected; mainly after the antibiotic exposure, and there was no difference in prevalence between the two groups. Thus, the probiotics did not influence the emergence of beta-lactamase in the microbiota. None of the analyzed samples were positive for ESBLs. The participants within this study were healthy adults, and since ESBLs are mostly prevalent in nosocomial settings\cite{20}, this may explain the absence of ESBLs.

The limited disturbance of the faecal microbiota correlates well with the limited gastrointestinal complaints reported by the volunteers. While a significant increase in various symptoms was reported; these did not exceed a level of slight discomfort; Bristol stool scale values remained in the normal range and the number of passed stools did not reach the level defined for diarrhoea which is 3 or more loose stools per day. The general mild symptoms could be explained by a relatively short exposure and low dose of antibiotics.

In conclusion, consumption of amoxicillin and clavulanate by healthy volunteers caused only minimal microbiota disturbances. Probiotic consumption lead only to small increased faecal levels of the consumed genera and species.

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**COMMENTS**

**Background**

Antibiotics have the potential to disturb the intestinal microbiota. This disturbance is one of the causes of antibiotic associated diarrhoea (AAD). Probiotics have been shown to reduce the risk of AAD. The mechanism is thought to be by stabilisation of the microbiota, but this has been little investigated.

**Research frontiers**

Studying antibiotic induced changes in the faecal microbiota composition have
been investigated only to a limited extent with molecular techniques as has the effect of probiotics on the microbiota.

**Innovations and breakthroughs**

To study the effect of probiotics on antibiotic induced changes in the faecal microbiota, a challenge model was used where healthy volunteers under defined conditions were exposed to antibiotics and probiotics or placebo in a randomised and blinded study set up.

**Applications**

Probiotics have been documented to reduce the risk for AAD. However, contrary to the common perception, the tested antibiotic (amoxicillin-clavulanate) appeared to cause only limited disturbance of the intestinal microbiota and hence the effect of probiotics on this was limited. Probiotics may therefore work through a different mechanism on AAD. The administered species are found to appear to cause only limited disturbance of the intestinal microbiota and hence the effect of probiotics on this was limited. Probiotics may therefore work through a different mechanism on AAD. The administered species are found to

**Terminology**

Probiotic: live microorganisms which when administered in adequate amounts confer a health benefit on the host. Microbiota: the microflora (and microfauna) in an ecosystem (usually an animal host or a single part of its body, such as intestines, mouth, vagina, etc.). Antibiotic associated diarrhoea results from an imbalance in the colonic microbiota caused by antibiotic therapy causing an osmotic diarrhea or allowing the overgrowth of potentially pathogenic organisms.

**Peer review**

It may be worth to be published because of all the uncertainties around the use of probiotics to prevent gastrointestinal disorders related to antibiotic treatments.

**REFERENCES**

1. Pérez-Cobas AE, Gosalbes MJ, Friedrichs A, Knecht H, Arta-acho A, Eismann K, Otto W, Rojo D, Bargiela R, von Bergen M, Neuling SC, Däumer C, Heinzen FA, Latorre A, Barbas C, Seifert J, dos Santos VM, Ott SJ, Ferrer M, Moya A. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. Gut 2013; 62: 1591-1601 [PMID: 23260099 DOI: 10.1136/gutjnl-2012-303184]

2. Food and Agriculture Organization/World Health Organ-ization. Guidelines for the evaluation of probiotics in food 2002. Available from: URL: http://www.who.int/foodsafety/publications/ls_management/probiotics2/en/ 1-11

3. Corr SC, Hill C, Gahan CG. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. Adv. Food Nutr Res 2009; 56: 1-15 [PMID: 19389604 DOI: 10.1016/S0063-4526(08)06001-3]

4. Engelbrektson A, Korzenik JR, Pittler A, Sanders ME, Kl-enhammer TR, Leyer G, Kitts CL. Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy. J Med Microbiol 2009; 58: 663-670 [PMID: 19695300 DOI: 10.1099/jmm.0.47615-0]

5. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, Johnsen B, Shekelle PG. Probiotics for the pre-vention and treatment of antibiotic-associated diarrheaa: a systematic review and meta-analysis. JAMA 2012; 307: 1959-1969 [PMID: 22570464 DOI: 10.1001/jama.2012.15207]

6. Lahinen SJ, Forsten S, Aakkio J, Granlund L, Rautonen N, Salminen S, Viitanen M, Ouwehand AC. Probiotic cheese containing Lactobacillus rhamnosus HN001 and Lactobacil-lus acidophilus NFCM® modifies subpopulations of fecal lactobacilli and Clostridium difficile in the elderly. Age (Dordr) 2012; 34: 133-143 [PMID: 21264685 DOI: 10.1007/s11357-011-0298-6]

7. Ouwehand AC, ten Bruggencate SJ, Schonewille AJ, Alho-niemi E, Forsten SD, Boeve-Oudenhoven IM. Lactobacillus acidophilus supplementation in human subjects and their

resistance to enterotoxigenic Escherichia coli infection. Br J Nutr 2014; 111: 465-473 [PMID: 23930950 DOI: 10.1017/ S0007114513002547]

8. Makivuokko I, Nurmi J, Nurminen P, Stowell J, Rautonen N. In vitro effects on polydextrose by colonic bacteria and caco-2 cell cyclooxygenase gene expression. Nutr Cancer 2005; 52: 94-104 [PMID: 16091099]

9. Mäkeläinen H, Forsten S, Saarinen M, Stowell J, Rau-tonen N, Ouwehand AC. Xylo-oligosaccharides enhance the growth of bifidobacteria and Bifidobacterium lactis in a simulated colon model. Benefc Microbes 2010; 1: 81-91 [PMID: 21831753]

10. Ventura M, Reniero R, Zink R. Specific identification and targeted characterization of Bifidobacterium lactis from different environmental isolates by a combined multiplex-PCR approach. Appl Environ Microbiol 2001; 67: 2760-2765 [PMID: 11375192]

11. Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. De-velopment of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol 2004; 97: 1166-1177 [PMID: 15546407]

12. Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. Appl Environ Microbiol 2004; 70: 6459-6465 [PMID: 15528506]

13. Matsuda K, Tsuji H, Asahara T, Kado Y, Nomoto K. Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription-PCR. Appl Environ Microbiol 2007; 73: 32-39 [PMID: 17071791]

14. Apajalaiti JH, Kettunen H, Kettunen A, Holben WE, Nur-minen PH, Rautonen N, Mutanen M. Culture-independent microbial community analysis reveals that inulin in the diet primarily affects previously unknown bacteria in the mouse cecum. Appl Environ Microbiol 2002; 68: 4986-4995 [PMID: 12524348]

15. Dimenäs E, Gisle H, Hallerbück B, Hernqvist H, Svedlund J, Wiklund I. Well-being and gastrointestinal symptoms among patients referred to endoscopy owing to suspected duodenal ulcer. Scand J Gastroent 1995; 30: 1046-1052 [PMID: 8578162 DOI: 10.3109/00365529509101605]

16. Dimenäs E, Carlsson G, Gisle H, Israelsson B, Wiklund I. Relevance of norm values as part of the documentation of quality of life instruments for use in upper gastrointestinal disease. Scand J Gastroent Suppl 1996; 221: 8-13 [PMID: 9110389 DOI: 10.3109/00365529609095544]

17. Koh H, Lee MJ, Kim MJ, Shin JL, Chung KS. Simple di-nostic approach to childhood fecal retention using the Leech score and Bristol stool form scale in medical practice. J Gastron-teroent Hepat 2010; 25: 334-338 [PMID: 19817956 DOI: 10.1111/j.1440-1746.2009.06015.x]

18. Plummer SF, Garaiova I, Sarvootham T, Cottrell SL, Le Scouller S, Weaver MA, Tang J, Dee P, Hunter J. Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy. Int J Antimicrob Agents 2005; 26: 69-74 [PMID: 15967639]

19. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal mi-crobiota. Microbiology 2010; 156: 3216-3223 [PMID: 20705661 DOI: 10.1099/mic.0.040618-0]

20. Ben-Ami R, Rodriguez-Baño J, Arslan H, Pitout JD, Quen-ting C, Calbo ES, Azap OK, Arpin C, Pascual A, Livermore DM, Garau J, Carmeli Y. A multinational survey of risk fac-tors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis 2009; 49: 682-690 [PMID: 19622043]
