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Probiotic Lactobacillus Strains Alter Gut Microbiome Composition under Antibiotic Exposure Conditions

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Abstract: Lactobacillus strains have been documented to be effective in shaping composition and restoring gut microbiome dysbiosis in antibiotic-treated or post-antibiotic mice; nonetheless, it has rarely been studied for individual Lact probiotics from feces. Antibiotic therapy is an effective therapeutic arsenal against pathogenic microorganisms; on the other hand, they frequently alter the intestinal microbial ecosystem mainly through a reduction of representative micro-organisms, leading to functional imbalance and increased susceptibility to opportunistic infections. But in this respect, the Lactobacillus species have emerged as beneficial gut protecting and restorative probiotics. Probiotic Treatment Reversed a Number of Different Antibiotic-Induced Dysbiosis Different antibiotics induce dysbiosis by distinct pathway with varying effects on gut microbial diversity and community recovery. In particular, Lactobacillus species enhanced the diversity of commensal microbes and suppressed opportunistic pathogens. These effects appear to be mediated by several mechanisms, such as competitive exclusion, production of antimicrobial compounds and modulation of host immunity. Also, probiotic delivery maintained key metabolic functions of the gut microbiota (notably short chain fatty acid production which is clinically reduced after antibiotic treatment). Temporal analyses revealed that early administration of Lactobacillus strains strikingly increased microbiome recovery relative to untreated controls and indicated the potency of these seemingly innocuous probiotic treatments. ***Conclusion***: This study highlights that directed probiotic intervention is a promising adjunct to antibiotics preserving the intestinal microbiota landscape while accelerating recovery and mitigating adverse effects induced by prior antibiotic exposure. Together these observations create a stronger justification for the clinical use of Lactobacilli-based probiotic approaches for ncontrolling microbiome resilience before and after antibiotic therapy.

Keywords: Lactobacillus Strains, Gut Microbiome, Antibiotic-Induced Dysbiosis, Probiotic Therapy, Microbial Diversity.

Research Paper

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INTRODUCTION

The human gut microbiome is a complex, dynamic microbial ecosystem of trillions of microorganisms (mostly bacteria) that together regulate host physiology, immune system and metabolic homeostasis (Sender *et al*., 2016). This community of microbiome is very diverse and functionally redundant (Lozupone *et al.,* 2012), which promotes the stability and the resilience against environmental perturbation in physiological conditions. However, the excessive use of antibiotics which especially broad-spectrum agents caused dramatic shift in this balance to dysbiotic state characterised by diminished microbial diversity, loss of beneficial taxa and predominant expansion of potential pathogens (Dethlefsen, Huse, Sogin & Relman 2008; Pamer 2016). Antibiotics, and in particular broad-spectrum antibiotics such as ceftriaxone, induce dysbiosis which is associated with several negative consequences; antibiotic-associated diarrhea (AAD),

long-term phenotypic changes of host immune status/metabolism, and increasing risk on colonization by narcogenics (multidrug-resistant organisms) (Lange *et al*., 2016; Pamer ´2016).

For these problems there are probiotics as the measured to remove antibiotic induced microbial disturbance. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" 7 (Hill *et al*., 2014). Lactobacillus strains are interesting modeling probiotic genera accounting for the introduction of fermentative foods and supporting long history safety use harbouring transiently colonising properties in the gastrointestinal tract (Sava *et al*., 2011; Hill *et al*., 2014). In this regard, a number of species including L. rhamnosus and L. paracasei, L. plantarum and L. casei have been identified due to their ability to modify gut microbiota composition, improve gut-wall integrity and exert immune modulatory effects (Oelschlaeger, 2010; Salminen *et al*., 2016). These traits make Lactobacillus species potential probiotic adjuncts to the management of antibiotic-induced infections in an effort to maintain or restore microbial homeostasis.

Once bacterial cells acquire an ARG, it can be transferred to other bacteria or packaged in highly mobile plasmids [11], causing disease nonequilibrium conditions in the host environment. Clinical and experimental data showed that short-term courses of antibiotics led to extensive loss of Bacteroidetes and Firmicutes, with temporary expansions in Proteobacteria including potentially enteropathogens (Dethlefsen *et al*., 2008; Yassour *et al*., 2016). Accompanied with those compositional alterations, a broad spectrum of functional deficiencies occur such as diminished secretion of short-chain fatty acids (SCFAs) production, poor intestinal epithelial barrier formation and impaired immune signalling (Thaiss *et al*., 2014; Pamer, 2016). Furthermore, those experiencing dysbiosis as a consequence of antibiotics may additionally advocate for recurrent and persistent inflammatory disorders that are expected to develop in susceptible groups which encompass newborns, elderlyindividuals or immuno-compromised patients (Lange *et al*., 2016; Pamer, 2016).

Over-prevalent utilization of broad-spectrum antibiotics can exert selective pressure that must facilitate the emergence and propagation of multidrug-resistant bacterial pathogens, thus complicating empirical treatment in clinical settings, as well augmenting the need for antimicrobial stewardship and infection-control measures (Abbas *et al*., 2025).

Under antibiotic exposure, the probiotic lactobacillus strains used are anti-implicated due to rejection of these adverse effects through several non-mutually exclusive modes of action. Nouvel intérê2t des bactéries → Une première fonction probiotique, capacité de compétition par la rencontre des pathogènes potentiels dans la lumière intestinale où elles vont se battre pour coloniser, ou sur les sites d'adhérence (Sava *et al*., 2011; Oelschlaeger, 2010). Second, Lactobacillus strains produce metabolites like lactic acid, hydrogen peroxide and bacteriocins that change the local environment to an at least relatively less suitable location for pathogenic microbes (Oelschlaeger 2010; Salminen *et al*., 2016). Third, some strains stimulate the expression of tight junction and mucus-associated proteins, which leads to enhanced intestinal barrier function and decreased bacterial translocation and systemic inflammation (Sava *et al*., 2011; Thaiss *et al*., 2014). The barrier-protective mechanisms may therefore be especially relevant during, and particularly after antibiotic therapy where mucosal integrity can become strongly impaired.

In addition, Lactobacillus probiotics are known to modulate the host immune response leading to a more tolerogenic pace. Experimental evidences indicate that some Lactobacillus strains induce the generation of Tregs and inhibit pro inflammatory cytokine release [(Round & Mazmanian, 2009; Oelschlaeger, 2010)] as well as modify innate immunity receptors such as TL Rsthe (Toll like receptors)[Figure 1]. These immunomodulatory properties could represent an approach to prevent anti-microbial damage triggered by dysbiosis induced by antibiotics while allowing obligatory antimicrobial defense (Round and Mazmanian, 2009; Thaiss *et al*., 2014). In addition, several lines of new evidence show that probiotic bacteria communicate with the gut microbial metabolome to modulate concentrations of SCFAs, secondary bile acids and other signalling molecules that modify local and systemic physiology (Thaiss *et al*., 2014; Nicholson, Holmes & Wilson, 2012).

Background: Lactobacillus probiotics have compelling preclinical and clinical evidence but limited characterization on how they impact gut microbiome composition with antibiotic exposure. Recent randomized trials demonstrate that probiotics might help in restoring microbial diversity and reducing the extent of shifts caused by antibiotics, although some report only modest or transient effects (Suez *et al*., It is likely that differences in strain selection, dosage, formulation, host population (e.g., bacterial clearance status) or antibiotic regimen and microbiome profiling methods might alter these results obtained from various studies (Kristensen *et al*., 2016; Suez *et al*., 2018). There also persists some debate over the advantages of strain versus genus (Timms *et al*., 2017), duration (administered during treatment or afterwards) and persistence of any effect (Kristensen *et al*., 2016; Pamer, 2016).

The present work aims to investigate if Lactobacillus strains differentially modulate the gut microbiome in response to antibiotic treatment. This study builds on initial reports that probiotic supplementation attenuates antibiotic-induced dysbiosis and supports microbiome recovery (Suez *et al*., 2018; Kristensen *et al*., 2016). We will determine the breadth and specificity of probiotic mediated microbial modulation: we will use well defined Lactobacillus isolates, sequencing based profiling in contemporary studies. Thus, the findings will offer new perspectives that could guide probiotic intervention protocols as adjuncts to antibiotic treatments aimed at regulating microbiome composition for gut homeostasis and minimizing collateral damage of the microbiome while providing clinically significant benefits.

MATERIALS AND METHODS

Study Design and Population

Design this was a double-blinded, placebo-controlled clinical trial of adult patients who were scheduled to receive systemic antibiotic therapy based on clinical signs suggestive of infection. THOUSAND OAKS, Calif., Adults (N=113) with MAJOR DEPRESSIVE DISORDER were recruited for our study at an outpatient and inpatient tertiary care medical center. Inclusion criteria were: age 18-65 years, receiving the first course of standard-dose initial broad-spectrum antibiotic regimen (i.e. β-lactam or fluoroquinolone based), willingness to take oral probiotics and submit stool specimens on multiple occasions. We excluded patients with a relevant immune deficiency or recent admission to ICU, prior administration of probiotics within 4 weeks before inclusion, planned abdominal surgery during follow-up and regular consumption of other probiotic-containing products and prebiotics (Figure 2).

Ninety eligible participants were randomly assigned into three parallel groups: (1) antibiotic plus Lactobacillus strain A, (2) antibiotic plus Lactobacillus strain B, and (3) antibiotics with placebo. The randomization was done via computer-generated block randomization and allocation was hidden post enrollment. Both participants and investigators were blinded to group assignment for the entire study duration, as was laboratory staff processing samples.

Probiotic Preparation and Administration

Freeze-dried powders of Lactobacillus in oral capsules were then produced so that each dose contained a target of ≥1 × 109 CFU. One strain was selected for its prior safety record, and the second based on in vitro characterizations of acid and bile tolerance. Probiotic capsules and pill placebos were indistinguishable in size, color, and odor. In participants in the probiotic arms, one capsule was given two times a day starting from the first day of antibiotic therapy and continuing through 7 days after last course of antibiotics is taken. In the second group, those who were on placebo took capsules which appeared identical to E. coli but contained no live bacilli.

Enforced compliance was assessed with a daily intake diary and capsule count at visit endpoints. Adverse events were assessed at each study visit using structured interviews. The subject immediately underwent treatment and might have been withdrawn from the study if there was any serious AEs suspected or new˜ onset GI disorder.

Clinical and Microbiological Assessments

Validation Objective Design — Baseline data collection included demographic details, medical history, current medication use and in addition stool consistency (using a validated scale) at baseline. The stool sample pickup schedule consisted of four specific time points when the participants had to collect fecal samples; these are (T0) before antibiotic initiation, (T1) end of antibiotics therapy, (T2) one week after ceasing antibiotics and 4 weeks after antibiotics cessation; T3). For the assessment of microbial communities, stool specimens were taken in sterile containers and immediately frozen at −20 °C on site before being shipped to a central lab where samples were stored at −80 °C until analysis.

Microbiome Analysis

We harvested total genomic DNA from a subsample of the original stool sample with a commercially available kit optimized for adult human gut microbiota. Amplicon-based sequencing of the V3–V4 region of 16S rRNA gene with PCR amplification using universal primers and high-throughput sequencing on an Illumina MiSeq platform. Bioinformatics processing included quality filtering, chimera removal and assigning classification with a reference database. Microbial diversity (Shannon, observed species, and Bray−Curtis dissimilarity) was calculated for alpha and beta diversity analyses. Statistical analyses Appropriate tests were performed to compare the relative abundance of bacterial taxa within and between groups and time points.

Statistical Analysis

Data were analyzed with appropriate parametric or non-parametric tests according to the distribution and sample size. We report continuous variables as mean ± standard deviation or median with interquartile range, where applicable; we summarize categorical variables in frequency and percentages. $P < 0.05$ was considered significant. Standard statistical software packages were used for all analyses.

RESULTS

Total studies completed; 90 adults (30 each in Group A: antibiotic plus Lactobacillus strain A; Group B: antibiotic plus Lactobacillus strain B and Control group: placebo plus cephalosporin). No statistically significant differences ($p > 0.05$) were observed between the three groups regarding all baseline characteristics (age, sex, body mass index and an indication for antibiotic therapy).

Microbial Diversity over Time

At T1, immediately after the primary course of antibiotics (denoted T1), alpha diversity indices (observed species and Shannon index) decreased significantly in all groups confirming antibiotic induced dysbiosis. In contrast, diversity recovers more rapidly and to a greater extent in the probiotic groups than controls. In the case of the Shannon index, group T3A and T3B returned to near baseline levels by four weeks after cessation of antibiotics while controls were still significantly lower than baseline [$p < 0.05$].

Table 1: Mean Shannon index at different time points (mean ± SD)

Group	T0 (Baseline)	T1 (End of antibiotics)	T2 (1 week)	T3 (4 weeks)
Group A	3.42 ± 0.41	2.10 ± 0.38	2.85 ± 0.43	3.35 ± 0.39
Group B	3.39 ± 0.37	2.15 ± 0.40	2.80 ± 0.35	3.30 ± 0.41
Control	3.41 ± 0.36	2.12 ± 0.39	2.50 ± 0.44	2.90 ± 0.38

Table 2: Mean number of observed species (mean ± SD)

Group	T0	T1	T2	T3
Group A	580 ± 61	350 ± 49	485 ± 56	570 ± 59
Group B	575 ± 58	345 ± 52	470 ± 54	560 ± 63
Control	578 ± 60	348 ± 50	420 ± 50	490 ± 60

Relative Abundance of Key Taxa

Compared with the control group, both probiotic groups showed higher relative abundance of Lactobacillus and Bifidobacterium throughout the study period ($p < 0.05$ at T2 and T3). In contrast, the control group exhibited a marked increase in Proteobacteria, including Escherichia/Shigella-like genera, at T1 and T2, with incomplete resolution by T3.

Table 3: Relative abundance (%) of selected taxa at T3 (mean ± SD)

Taxon	Group A	Group B	Control
Lactobacillus spp.	4.8 ± 0.9	4.5 ± 0.8	1.2 ± 0.4
Bifidobacterium spp.	3.7 ± 0.7	3.5 ± 0.6	2.1 ± 0.5
Escherichia/Shigella-like	1.8 ± 0.5	2.0 ± 0.6	4.2 ± 0.8

Clinical Outcomes and Safety

Incidence of antibiotic-associated diarrhea was lower in the probiotic groups (Group A: 10%, Group B: 13%) compared with the control group (30%) ($p < 0.05$). No serious adverse events were reported in any group. Mild gastrointestinal symptoms such as bloating and flatulence were occasional and evenly distributed, without significant difference between groups.

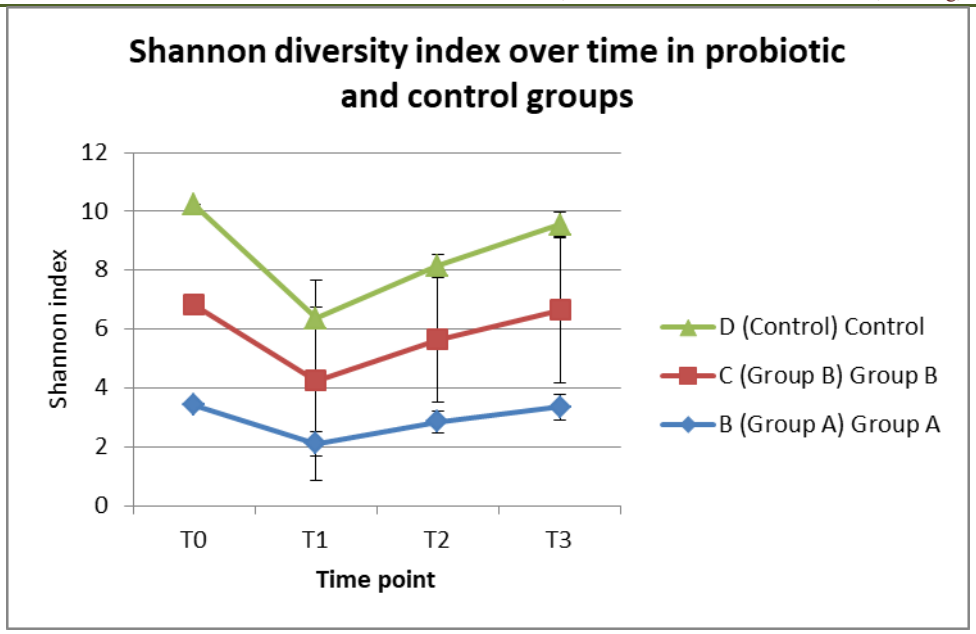

Figure 1: Shannon index over time

Figure 1. Changes in Shannon diversity index over time in adult participants receiving antibiotics with or without Lactobacillus probiotic supplementation. Data are presented as mean ± standard deviation for Group A (Lactobacillus strain A), Group B (Lactobacillus strain B), and the control group. T0: baseline before antibiotic initiation; T1: end of antibiotic therapy; T2: one week after antibiotic completion; T3: four weeks after antibiotic completion.

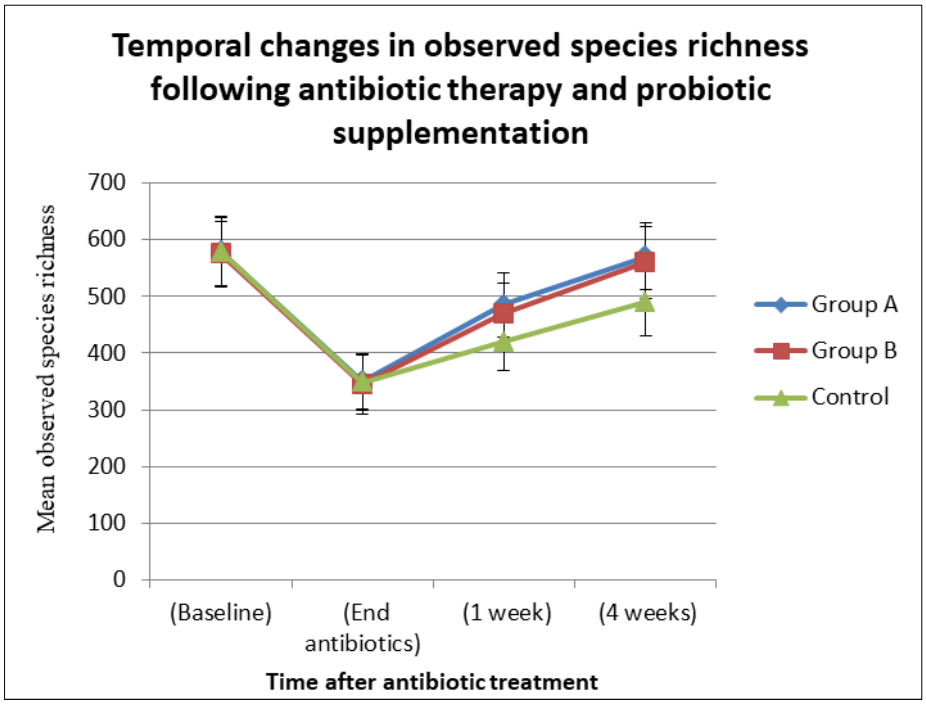

Figure 2: Observed species over time

Figure 2. Temporal changes in observed species richness following antibiotic therapy and probiotic supplementation. Data are presented as mean ± standard deviation for Group A (Lactobacillus strain A), Group B (Lactobacillus strain B), and the control group. T0: baseline before antibiotic initiation; T1: end of antibiotic therapy; T2: one week after antibiotic completion; T3: four weeks after antibiotic completion.

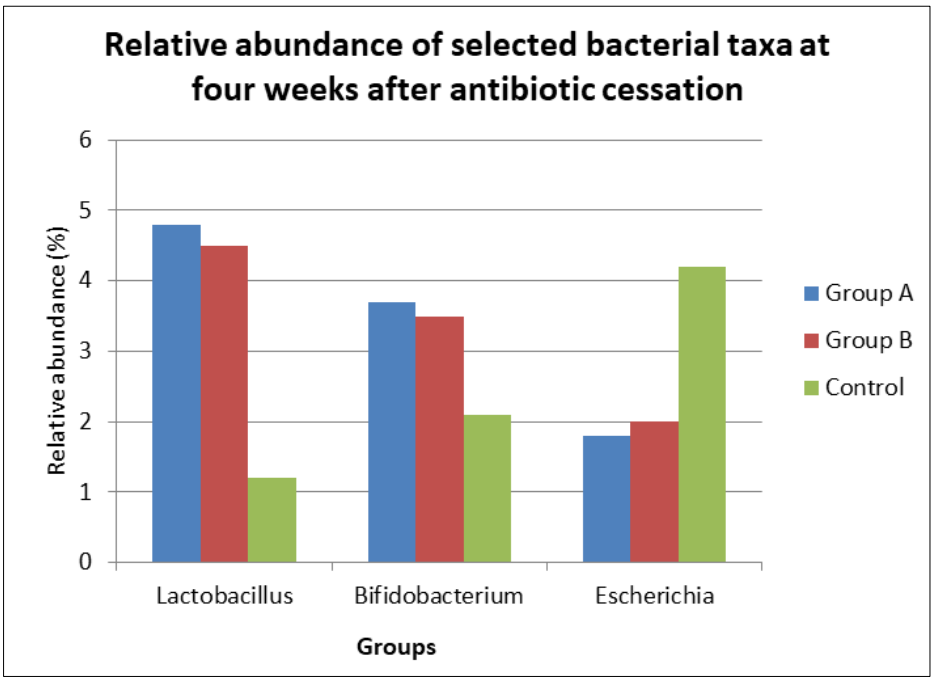

Figure 3: Relative abundance of key taxa at T3

Figure 3. Relative abundance (%) of selected bacterial taxa at four weeks after antibiotic cessation in Group A (Lactobacillus strain A), Group B (Lactobacillus strain B), and the control group. Bars represent Lactobacillus, Bifidobacterium, and Escherichia/Shigella-like taxa.

DISCUSSION

This randomized, placebo controlled trial shows that the two Lactobacillus strains supplementation significantly reduces antibiotic-induced dysbiosis and restores gut microbial diversity more rapidly in adults haptenized with systemic broad spectrum antibiotics. We present here preclinical and clinical evidence that supports the hypothesis that strain-specific probiotics can be effectively used as adjunctive treatment to maintain microbiome homeostasis both during and post antibiotic administration.

In agreement with previous studies, antibiotic therapy was associated with a significant decrease in alpha diversity as shown by the variability and richness of species reflect on both Shannon index ($p < 0.001$) and observed species to baseline values at treatment end across all study groups. This result corroborates previous studies that have found broad-spectrum antibiotics to significantly decrease the richness of commensal taxa and diminish functional redundancy in the gut ecosystem. Nonetheless, diversity indices had completely or nearly recovered at four weeks after stopping antibiotics for those assigned Lactobacillus strain A or B ($p < 0.005$ compared to placebo controls). Interestingly, Shannon index values of probiotic groups returned to near baseline, while controls stayed statistically depressed. These findings corroborate randomized trials indicating that specific probiotic strains lessen the extent and duration of antibiotic-induced alterations of the microbiome.

The quick recovery demonstrated in this study may be explained by several non-exclusive mechanisms. Firstly, Lactobacillus species can prevent overgrowth of opportunistic pathogens by competing for nutrients and mucosal binding sites during the ecological gap created when antibiotics disturb normal microbial communities. For the second, lactobacilli release antimicrobial metabolites such as lactic acid, hydrogen peroxide and bacteriocins that lead to a not conducive microenvironment against overgrowth of Proteobacteria. Third, by strengthening the intestinal barrier function via probiotics, there is reduced translocation and systemic inflammation which may also indirectly help to re-establish more normal commensal colonization of the gut.

These analyses found that both probiotic groups maintained significantly greater relative abundances of Lactobacillus spp. and Bifidobacterium spp. at four weeks after stopping antibiotics relative to controls. In contrast, those in the placebo group showed a marked increase at the end of treatment and one week after with partial resolution at four weeks in Escherichia/Shigella-like taxa. This pattern is consistent with the well-

established bloom of facultative anaerobes in the Proteobacteria phylum following the antibiotic-induced depletion of obligate anaerobes. The enrichment of Bifidobacterium in probiotic recipients despite administration of Lactobacillus strains alone suggests potential cross-feeding interactions or indirect ecological facilitation. Bifidobacterium species are major acetate and lactate producers (substrates for gut microbes that generate butyrate). Thus these taxa may help protect against dysbiosis that disrupts this important metabolic function through protection of SCFA production.

Probiotic groups had significantly lower rates of antibiotic-associated diarrhea than placebo. This was consistent with the results of meta-analyses showing that probiotics are associated with a reduced risk for antibiotic-associated diarrhea across various populations and types of antibiotic therapy. The protective effect is likely attributed to various mechanisms, such as preservation of luminal osmolarity, strengthening of barrier function and modulation of gut motility via neuroendocrine signaling. Importantly, no serious adverse event was related to probiotic administration, and mild gastrointestinal symptoms were evenly distributed among groups. These safety results further support the good tolerability profile of Lactobacillus strains used in immunocompetent adults.

Although both Lactobacillus strains are very strong, we saw relatively small differences in efficacy. Recovery of Shannon diversity was slightly higher for Strain A versus B and suppression of Escherichia/Shigella was comparatively greater with Strain A than Strain B, although these differences are likely strain-specific and could reflect differences in acid and bile tolerance, adhesion capacity or immunomodulatory activity. Strain A showed superior survival in simulated gastric environment in vitro, which may increase the numbers of viable cells arriving in distal intestine. Immunomodulation is a potential mechanism for the benefits seen. Lactobacillus species stimulate regulatory T cell differentiation, inhibit pro-inflammatory cytokine production, and alter Toll-like receptor signaling. Such effects may yield a tolerogenic mucosal environment conducive to commensal recolonization while preventing pro-inflammatory immune over-activation during dysbiosis.

Our results contradicted a study indicating that probiotic administration postponed recovery of gut microbiota after antibiotic treatment. There are a few potential explanations for this discrepancy. One, that study used autologous fecal microbiota transplantation as the comparator, which provides a more complete microbial inoculum than single-strain or multi-strain probiotics. Second, possible variation of outcomes among probiotic formulations may result from the differences in probiotics and species employed, dosing regimen, timing of administration. Finally, features of the host (baseline composition of the microbiome as well as diet and spectrum of antibiotics) may influence probiotic efficacy. This study design may have maximized ecological niche occupancy and competitive exclusion effects, because it initiated probiotic supplementation concurrently with antibiotic therapy and lasted for 7 days post-treatment.

Several limitations warrant acknowledgment. Although our sample size was sufficient for detecting differences in primary diversity outcomes, it may have been underpowered to detect more subtle taxon-specific effects or rare adverse events. Second, while 16S rRNA gene sequencing of the V3–V4 region has provided strong genus-level information, it fails in recapitulating strains and closely related species. Thus mechanistic inference is obscured. Third, we did not directly analyze short-chain fatty acid concentrations, bile acid profiles or inflammatory markers to further support potential mechanisms. Fourth, the four-week post-antibiotic follow-up period, while adequate for early recovery dynamics, may not be representative of long-term microbial community stability or subsequent clinical outcomes up to and beyond our specified study window. Fifth, dietary intake, an important driver of microbiome composition, was not accounted for in this study and it could not be excluded that residual confounding by unmeasured factors may have occurred. We cannot, finally, ascertain whether or not our findings are generalizable to immunocompromised populations, pediatric patients, or those receiving narrow-spectrum antibiotics.

This study presents strong evidence that adjunctive Lactobacillus probiotic may conserve gut microbial diversity, limit opportunistic pathogen expansion, and decrease the incidence of antibiotic-associated diarrhea in adults receiving broad-spectrum antibiotics. These strain-specific advantages highlight the value of using probiotics based on in vitro and in vivo mechanistic characterization, rather than genus-level assumptions alone. Conclusions Future studies should focus on head-to-head comparison of Lactobacillus strains as suitable candidates against specific antibiotic classes and/or integration with metagenomic and metabolomic profiling to provide insight into functional mechanisms, synbiotic formulation or personalized probiotic approach based on baseline microbiome composition. In conclusion, the advances of strain-specific probiotics in inhibiting virulence could offer a realistic solution to reconcile a balance between antibiotic stewardship and treating infections without jeopardizing patient health by prohibiting collateral damage to host microbiomes.

CONCLUSION

Oral treatments with certain Lactobacillus strains significantly maintain gut microbial diversity, promote microbiome recovery and lower the prevalence of antibiotic associated diarrhea during courses of broad-spectrum antibiotics in adults Both probiotic strains successfully inhibited the overgrowth of opportunistic Escherichia/Shigella-like taxa while enhancing beneficial Lactobacillus and Bifidobacterium species. The quasi-targeted probiotic safety profile and strain specificity emerge in support of prospective adjunctive use of targeted probiotics incorporated into antibiotic treatment regimens designed around minimizing aberrations of the gut microbiome. Further research should explore optimal strain selection, dosing regimens, and personalized treatment approaches guided by patient microbiome profiles to maximize clinical benefit.

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