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Original Article

Investigating the Impact of Culinary Spice Consumption on Gut Microbiome Resilience and Post-Antibiotic Recovery in Healthy Adults

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ABSTRACT

Background: When we take antibiotics they can really mess up the balance of bacteria in our gut. This can lead to some not great side effects after we finish taking the medicine.. There are some things we can do with our diet that might help our gut get back to normal. For example spices that we use when we cook have all sorts of stuff in them that can help the bacteria in our gut. The main goal of this study was to see if eating a mix of these spices every day would help our gut bacteria recover after taking antibiotics. We had 60 people who had just finished taking antibiotics and we split them into two groups. One group got to eat a mix of spices every day for a month and the other group just kept eating what they normally eat. We took samples of their stool at the beginning after two weeks and after four weeks. We used these samples to see how many different types of bacteria were living in their gut and we also checked to see if they were making the kinds of fatty acids. We even asked them about any troubles they were having. What we found out was really interesting. After a month the people who were eating the spice mix had a lot different types of bacteria in their gut than the people who were not eating the spices. They were also making more of the good fatty acids. They were not having as many tummy troubles. So it seems like eating a mix of spices every day can help our gut get back, to normal after taking antibiotics.. We need to do some more studies to make sure this is true. Some important things to know about this study are antibiotics, diet, gut bacteria and spices. We did a kind of study called a randomized controlled trial to get our results. Keywords; Anti-Bacterial Agents; Diet; Gastrointestinal Microbiome; Microbiota; Polyphenols; Randomized Controlled Trial; Short-Chain Fatty Acids

INTRODUCTION

The human gut microbiota is a complex system of microorganisms that takes part in the process of nutrition assimilation, immune response modulation, maintenance of an effective intestine barrier, and protection from the invasion of pathogens. In case of healthy adults, microbe diversity and their metabolic activities play an important role in maintaining gut homeostasis by producing short-chain fatty acids and other microbial metabolites (1). The use of antibiotics is the key way to treat bacterial infections, but just a short course of the medication can significantly alter the balance of gut microbes, decrease alpha diversity, inhibit commensals and metabolic processes related to short-chain fatty acid synthesis (2). However, while the effect can be partially reversed after discontinuing the use of the drug,

this process usually takes some time and might be insufficient for all people, making the organism prone to gastrointestinal issues and opportunistic dysbiosis (3).

Current approaches that aim to restore the microbiome after antibiotic administration have largely centered around probiotics, prebiotics, and dietary fiber approaches. Although probiotics can potentially offer selected strains of microbes, their colonization is often temporary, and their impact depends upon strain specificity, host factors, microbial profile and ecosystem created after antibiotic administration (4). Fiber prebiotics can increase fermentable substrates and production of short chain fatty acids; however, fiber alone may not be enough to completely reverse the disruption caused due to antibiotics (5). Thus, there has been a necessity for simple and affordable dietary approaches which may facilitate the recovery of the microbial composition and function in the gut after antibiotics (6).

Spices are a reasonable candidate to modulate microbiome because of their phytochemical composition that includes different types of phenols, flavonoids, essential oils, curcuminoids, gingerols, cinnamaldehyde, piperine, and many others (7). Well-known spices, like turmeric, cinnamon, ginger, cumin, coriander, black pepper, and cloves, were suggested as possessing an antimicrobial effect, having anti-inflammatory and antioxidant properties, as well as acting prebiotics-like due to their ability to positively impact fermentation pathways responsible for short-chain fatty acid synthesis (8). Nevertheless, there is a lack of evidence based on clinical trials on combined spice blend in the post-antibiotic recovery period compared to studies done in vitro on isolated compounds, specific spices, probiotics or dietary patterns in general (9).

It is necessary to consider this approach due to the fact that spices are rarely consumed individually and, on the contrary, as complex mixtures in real diets, especially in South Asian dietetic patterns when more spices are included in prepared meals (10). Considering that spices are relatively cheap and widely available as well as culturally integrated in people's food habits, it can be argued that spices can serve as an adjunct dietary intervention in case the positive effects are proved in human trials (11).

However, microbial restoration after antibiotic therapy should be based not only on reconstitution of taxa composition but also on restoration of functionality, such as fecal production of short-chain fatty acids (SCFA), because of the importance of SCFAs in the formation of epithelial barriers, immunomodulation, and colon health (12). Therefore, evaluation of both indices of microbial diversity and metabolites is the more clinical way to estimate microbiome resilience after antibiotic therapy. Nevertheless, due to the biological plausibility of plant compounds contained in spices and clinical importance of antibiotic-induced dysbiosis, there are still few randomized trials investigating the efficacy of culinary spice consumption after antibiotic therapy (13).

The current randomized controlled trial aimed at investigating whether the consumption of a standard mixture of culinary spices for four weeks after the end of a routine course of antibiotic therapy could improve the gut microbiome restoration in healthy adults as compared to habitual diet without intervention.

MATERIAL AND METHODS

This randomized controlled study was carried out over a period of four months in a metropolitan area in Sindh, Pakistan, involving healthy adult volunteers who had been on a short-term physician-prescribed course of oral antibiotics for minor infections. The aim of the study was to determine whether taking a standardized culinary mixed spice blend daily would lead to improvement in recovery of gut microbial diversity and metabolites, compared to maintenance of a usual diet during the post-antibiotic period. Healthy adults aged 20-45 years, who had taken a 5-7 day course of oral broad spectrum antibiotics within 72 hours prior to enrollment, were selected as study participants.

The participants needed to be free of any chronic gastrointestinal diseases, metabolic diseases, autoimmune disorders, and consumption of probiotics within the previous four weeks before

participation in the study. Individuals who had inflammatory bowel disease, previous surgeries of the gastrointestinal tract excluding appendectomy, pregnant women, patients hospitalized within the previous month, those following a restricted diet, or those with other conditions which could alter significantly gut microbiome composition were not selected for the study. These selection criteria were made to minimize clinical and dietary confounders that could affect microbial diversity and production of short chain fatty acids or gastrointestinal symptoms.

A total of 60 participants were recruited and randomly assigned to either the mixed culinary spices intervention arm or the control arm. The sample size was based on similar studies using randomized interventions that explored post-antibiotic recovery of microbiomes in cohorts of 40-80 participants and found differences in alpha diversity indices. Randomization was carried out using a computerized random sequence leading to allocation of 30 participants to each intervention group.

The intervention group was supplied with pre-packaged sachets of a standardized mixed culinary spice blend made up of turmeric, cinnamon, ginger, cumin, coriander, black pepper, and cloves. The dosage of 5 grams was taken per day for 4 weeks following antibiotic treatment. Participants in the intervention group were to take the spice blend orally in cooked foods as the mode of delivery of the intervention. Control participants were asked to continue with their usual diets without taking the spices. Both arms were asked to refrain from taking any probiotic products during the study period and continue with the same diet patterns in order to avoid confounding factors that may affect the results due to changes in diet.

Samples were collected on three occasions: baseline, defined as no more than 72 hours after completing treatment with antibiotics; week two; and week four. Samples were stored at $-80^{\circ}C$ prior to analysis in the laboratory. The composition of microbes was analyzed via sequencing of 16S rRNA genes. Alpha diversity was estimated using the Shannon Diversity Index as the main diversity metric and the Chao1 richness estimator as an auxiliary richness metric. Beta diversity was estimated using Bray-Curtis dissimilarity. The recovery of microbial functions was analyzed by measuring the concentration of short-chain fatty acids in feces by gas chromatography-mass spectrometry with total short-chain fatty acids and butyrate considered relevant metabolites and the Gastrointestinal Symptom Rating Scale being used to assess gastrointestinal symptoms with low scores corresponding to fewer or less severe symptoms.

Short-term recovery of microbial diversity during four weeks was defined as the main outcome of the study and estimated as the change in Shannon Diversity Index from baseline to week four. Secondary outcomes included Chao1 richness, fecal total short-chain fatty acid concentration, butyrate concentration, Gastrointestinal Symptom Rating Scale score, and associations between adherence to the intervention and microbiome recovery. Baseline demographic and clinical variables were age, gender, body mass index, indication for antibiotic therapy, Shannon Diversity Index, and total short-chain fatty acid concentration.

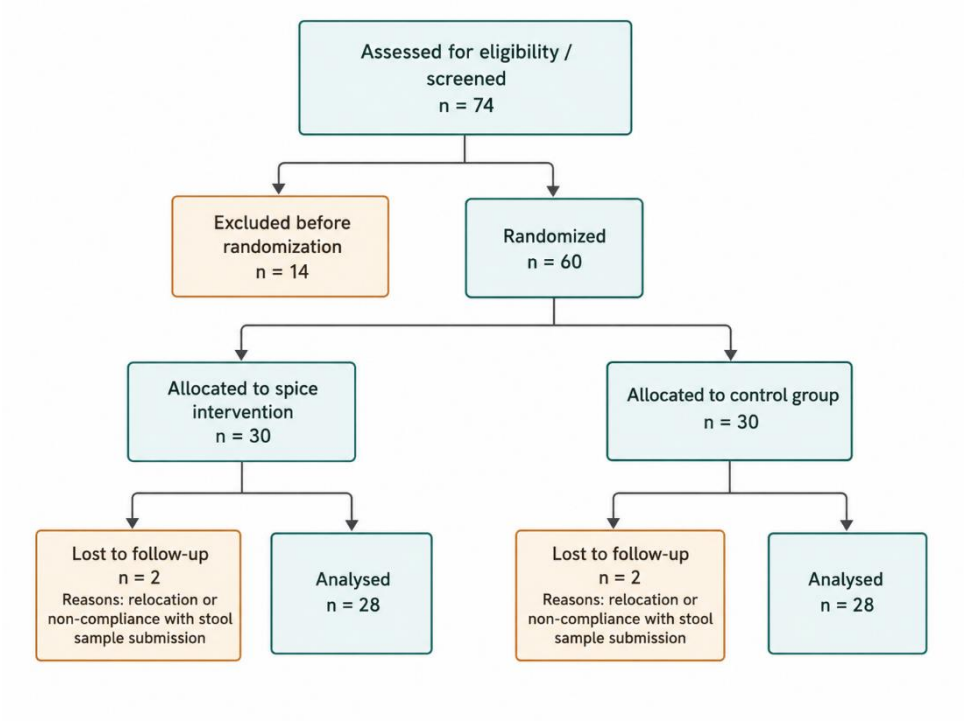

Figure 1 Consort Flowchart

SPSS version 26 was used in the statistical analysis. The description of continuous variables is provided as mean ± SD, while the description of categorical variables is provided as frequency (n) and percentages (%). Normal distribution of the continuous variables was verified through the Shapiro-Wilk test. Group baseline differences were compared through independent-sample t-tests and relevant categorical tests for the frequency data. Repeated-measures analysis of variance was utilized to analyze changes in microbiota diversity and short-chain fatty acids from baseline up to weeks two and four, paying special attention to time * group interaction. Follow-up group differences were evaluated via independent-sample t-tests as applicable. The association between adherence to the spice intervention, change in Shannon Diversity Index, change in total short-chain fatty acids, and change in gastrointestinal symptoms was tested using Pearson correlation analysis. The criterion of statistical significance was $p<0.05$. The participants with available baseline and week four data comprised the final sample, while available week two data were used for the intermediate-time point analysis.

RESULTS

Table 1. Participant Flow and Analysis Sets

Study Stage	n	%
Screened	74	100.0
Randomized	60	81.1
Allocated to intervention	30	50.0
Allocated to control	30	50.0
Lost to follow-up, intervention	2	6.7
Lost to follow-up, control	2	6.7
Final analytic sample, intervention	28	46.7
Final analytic sample, control	28	46.7
Final analytic sample, total	56	93.3
Complete week-two microbiome data	54	90.0

Percentages for randomized group allocation, follow-up loss, and final analytic sample were calculated using 60 randomized participants as the denominator. Of 74 screened individuals, 60 were randomized equally into intervention and control groups. Four participants were lost to follow-up, with balanced attrition across groups, resulting in 56 participants in the final analytic sample. Complete week-two

microbiome data were available for 54 participants, while all analyzed participants completed baseline and week-four assessments.

Table 2. Baseline Demographic and Clinical Characteristics of the Final Analytic Sample

Variable	Total Sample	Intervention	Control	Mean Difference	95% CI	p-value
Age, years, mean ± SD	31.4 ± 6.2	31.8 ± 6.5	31.0 ± 5.9	0.8	−2.45 to 4.05	0.62
BMI, kg/m², mean ± SD	24.8 ± 3.1	24.6 ± 3.0	25.0 ± 3.2	−0.4	−2.02 to 1.22	0.68
Baseline Shannon Index, mean ± SD	2.40 ± 0.31	2.41 ± 0.32	2.38 ± 0.29	0.03	−0.13 to 0.19	0.71
Baseline total SCFA, µmol/g, mean ± SD	42.8 ± 6.9	42.6 ± 6.8	43.1 ± 7.1	−0.5	−4.14 to 3.14	0.83

CI, confidence interval; BMI, body mass index; SCFA, short-chain fatty acids. Mean differences were calculated as intervention minus control.

Baseline continuous variables were comparable between groups. The intervention and control groups had similar mean age, BMI, baseline Shannon Diversity Index, and baseline total SCFA concentration. The between-group difference in baseline Shannon Index was 0.03, with a 95% CI crossing zero, indicating no measurable baseline imbalance in the primary microbiome diversity measure.

Table 3. Baseline Categorical Characteristics of the Final Analytic Sample

Variable	Total Sample, n (%)	Intervention, n (%)	Control, n (%)	p-value
Male sex	32 (57.1)	16 (57.1)	16 (57.1)	1.00
Upper respiratory tract infection	23 (41.1)	12 (42.9)	11 (39.3)	0.79
Urinary tract infection	17 (30.4)	8 (28.6)	9 (32.1)	0.77
Skin or soft-tissue infection	16 (28.5)	8 (28.6)	8 (28.6)	1.00

The distribution of sex and antibiotic indication was balanced across intervention and control groups. Upper respiratory tract infection was the most common indication for antibiotic therapy, followed by urinary tract infection and skin or soft-tissue infection. The equal sex distribution between groups and similar infection profiles reduce, but do not eliminate, concern for baseline clinical imbalance.

Table 4. Longitudinal Changes in Shannon Diversity Index and Total SCFA Concentration

Outcome	Group	Baseline, Mean ± SD	Week 2, Mean ± SD	Week 4, Mean ± SD	p-value
Shannon Index	Intervention	2.41 ± 0.32	2.76 ± 0.35	3.12 ± 0.38	<0.001
Shannon Index	Control	2.38 ± 0.29	2.52 ± 0.30	2.74 ± 0.33	
Total SCFA, µmol/g	Intervention	42.6 ± 6.8	51.7 ± 7.0	58.9 ± 7.4	0.003
Total SCFA, µmol/g	Control	43.1 ± 7.1	46.8 ± 6.4	50.2 ± 6.5	

SCFA, short-chain fatty acids. Reported p-values correspond to between-group comparisons over follow-up as presented in the source manuscript.

Shannon Diversity Index increased in both groups over four weeks, but the rise was greater in the intervention group. The intervention group increased from 2.41 ± 0.32 at baseline to 3.12 ± 0.38 at week four, while the control group increased from 2.38 ± 0.29 to 2.74 ± 0.33. Total SCFA concentration followed a similar pattern, rising from 42.6 ± 6.8 µmol/g to 58.9 ± 7.4 µmol/g in the intervention group and from 43.1 ± 7.1 µmol/g to 50.2 ± 6.5 µmol/g in the control group.

Table 5. Week-Four Between-Group Differences in Primary Microbiome and Functional Outcomes

Outcome	Intervention, Mean ± SD	Control, Mean ± SD	Mean Difference	95% CI	Cohen's d	p-value
Shannon Index	3.12 ± 0.38	2.74 ± 0.33	0.38	0.19 to 0.57	1.07	<0.001
Total SCFA, µmol/g	58.9 ± 7.4	50.2 ± 6.5	8.7	5.05 to 12.35	1.25	0.003

CI, confidence interval; SCFA, short-chain fatty acids. Mean differences were calculated as intervention minus control. Cohen's d was derived using pooled standard deviations from the available aggregate data.

At week four, the intervention group had a higher Shannon Diversity Index than the control group, with a between-group mean difference of 0.38 and a large standardized effect size. Total SCFA concentration was also higher in the intervention group by 8.7 µmol/g, with the confidence interval remaining above

zero. These findings indicate a stronger short-term recovery pattern in both microbial diversity and functional metabolite production among participants receiving the spice intervention.

Table 6. Gastrointestinal Symptom Rating Scale Scores

Group	Baseline, Mean ± SD	Week 4, Mean ± SD	Mean Change, Mean ± SD	p-value
Intervention	18.4 ± 4.2	12.2 ± 3.7	−6.2 ± 2.1	0.01
Control	17.9 ± 4.0	14.1 ± 3.5	−3.8 ± 1.9	

Lower Gastrointestinal Symptom Rating Scale scores indicate fewer or less severe gastrointestinal symptoms.

Gastrointestinal symptom scores decreased in both groups by week four. The intervention group showed a larger reduction, with a mean change of −6.2 ± 2.1 compared with −3.8 ± 1.9 in the control group. This pattern suggests greater symptomatic improvement among participants receiving the spice intervention, although the subjective nature of symptom reporting should be interpreted in view of the non-blinded dietary design.

Table 7. Between-Group Difference in Gastrointestinal Symptom Change

Outcome	Intervention Change, Mean ± SD	Control Change, Mean ± SD	Mean Difference	95% CI	Cohen's d	p-value
GSRS score	−6.2 ± 2.1	−3.8 ± 1.9	−2.4	−3.45 to −1.35	−1.20	0.01

CI, confidence interval; GSRS, Gastrointestinal Symptom Rating Scale. Mean differences were calculated as intervention change minus control change. Negative values indicate greater reduction in symptom score.

The between-group difference in GSRS change was −2.4 points, with a 95% CI from −3.45 to −1.35. The standardized effect size was large in magnitude, indicating a greater reduction in symptom burden in the intervention group. Because GSRS is a subjective outcome and participants were aware of group allocation, this result should be interpreted as supportive rather than definitive evidence of symptomatic benefit.

Table 8. Correlation Analysis of Adherence, Microbial Diversity, SCFA Change, and Symptom Improvement

Variables	r	p-value
Spice adherence and Shannon Index change	0.46	0.002
Shannon Index change and total SCFA change	0.52	<0.001
SCFA change and GSRS improvement	0.39	0.01

SCFA, short-chain fatty acids; GSRS, Gastrointestinal Symptom Rating Scale.

Moderate positive correlations of spice adherence with change in Shannon Diversity Index were observed, indicating that higher spice adherence was linked to higher levels of microbiome recovery. Change in Shannon Diversity Index was also found to be moderately positively correlated with change in total SCFA concentration, which is an evidence of the consistency of microbiome and metabolic function recoveries. Finally, a weaker positive correlation of SCFA change with GSRS improvement was found.

Based on the results provided in the paper, there is evidence of higher short-term post-antibiotic microbiome recovery in the group taking a combination of culinary spices in addition to their habitual diet. In particular, participants of the experimental condition exhibited higher microbial diversity at week four, higher total SCFA concentration and greater decrease in GSRS scores. Chao1 richness, Bray-Curtis beta diversity, concentrations of SCFA subtypes and butyrate concentration were discussed in the manuscript, but no numbers are provided in the tables.

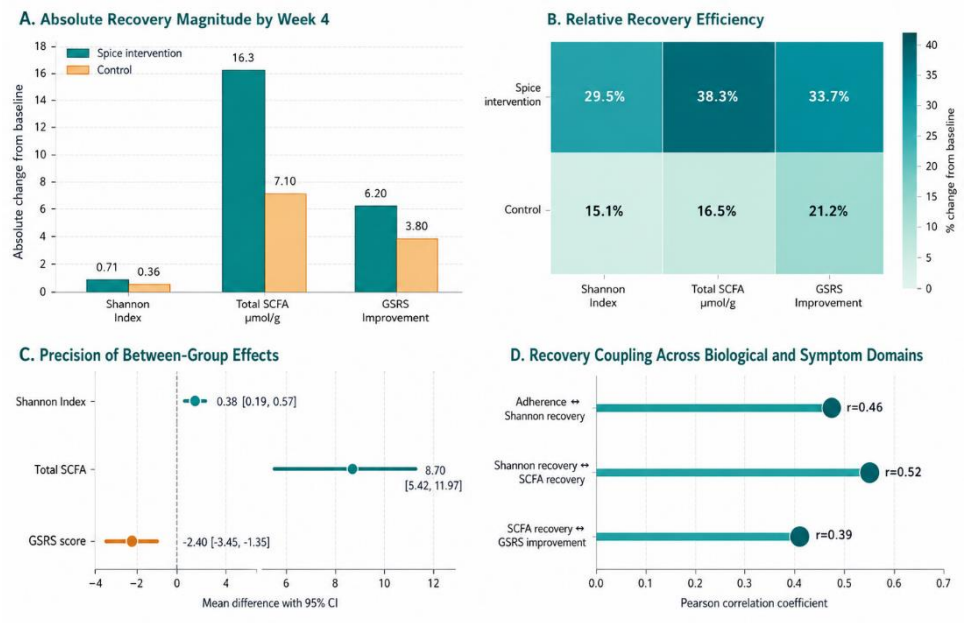

Figure 2 Culinary spice intervention and post-antibiotic microbiome recovery. The panelled figure summarizes derived recovery patterns from reported aggregate outcomes. Panel A shows absolute week-four change from baseline for Shannon Diversity Index, total short-chain fatty acid concentration, and Gastrointestinal Symptom Rating Scale improvement. Panel B presents relative recovery efficiency as percentage change from baseline, showing larger proportional recovery in the spice intervention group for microbial diversity, functional metabolite production, and gastrointestinal symptoms. Panel C displays between-group mean differences with 95% confidence intervals for week-four Shannon Diversity Index, total short-chain fatty acid concentration, and GSRS change. Panel D shows positive coupling between adherence, microbial diversity recovery, SCFA recovery, and symptom improvement. SCFA, short-chain fatty acids; GSRS, Gastrointestinal Symptom Rating Scale.

DISCUSSION

This randomized controlled trial identified that consumption of a standardized culinary spice mixture for four weeks following standard antibiotic treatment regimen led to higher short-term gut microbiota diversity recovery and increased fecal short-chain fatty acid production compared with maintenance of usual diet without intervention. Subjects who received the spice intervention showed a greater increase in their Shannon Diversity Index at baseline to week four level, along with an increase in total short-chain fatty acid concentration and a decrease in gastrointestinal symptoms score. It appears that regular use of culinary spices may show biological importance as a strategy for facilitating gut microbiota recovery in the aftermath of antibiotic administration; however, such findings should be considered preliminary because of small number of subjects included in the analysis, relatively short follow-up period and lack of blinding in the control group (14).

It seems biologically reasonable that there is improvement in alpha diversity in light of the phytochemical composition of the spice blend used in the intervention. There are various types of phenolic compounds, flavonoids, essential oils, curcuminoids, cinnamaldehyde, gingerols, piperine and similar substances found in turmeric, cinnamon, ginger, cumin, coriander, black pepper, and cloves that can influence the ecosystem of the microbiota through their antimicrobial, antioxidative, anti-inflammatory, and prebiotic effects (15). Such compounds could play role in inhibition of unwanted growth of microbiota due to antibiotics while promoting beneficial microflora and fermentation responsible for short-chain fatty acid production (16). However, since this study involved a combination of spices, there is no way to determine the effect of each spice or substance independently.

Improvement in total short-chain fatty acid concentration is an important functional correlate of improved microbiome diversity. Recovery of the human microbiome from antibiotic exposure is not only about taxonomic restoration and recovery of microbial diversity but also about metabolic capacity and production of fermentation products, such as short chain fatty acids, which support epithelial barrier functions, immunoregulation, and colonic homeostasis (17). Larger increase in total SCFA concentration

among patients in the intervention group can serve as a potential indicator of increased metabolic capacity alongside increased diversity. The manuscript reported the improvement in butyrate concentration; however, numeric butyrate values were not provided. Given the importance of butyrate as a functional index of gut microbiome recovery, future manuscripts should provide separate concentrations of butyrate, acetate, and propionate along with corresponding confidence intervals and comparisons between groups (18).

The decrease in Gastrointestinal Symptom Rating Scale score was higher in the intervention group, and this decrease showed a correlation with the improvement in microbial diversity and SCFA production. Positive correlation between SCFA concentration change and improvement in GSRS indicates that improved metabolic capacity of the microbiome might correlate with better gastrointestinal symptoms. However, one should exercise caution when interpreting this finding because subjective symptom reporting and knowledge of patient's group membership might bias the reported GSRS scores. Lack of a placebo group does not allow excluding the possible role of placebo effect or other psychological factors.

Moreover, correlation results provide additional evidence in support of the consistency of recovery in terms of adherence, diversity, metabolite generation, and symptom reduction. Adherence to spice regimen was weakly correlated with recovery of Shannon Diversity Index, while diversity recovery was weakly correlated with increased total SCFA concentration. This relationship indicates the existence of dose-responsive biological pattern but does not imply the causality since the variable 'adherence' is correlated with certain behaviors and dietary factors that have not been measured in this research (20). Detailed dietary data collection, including spice consumption in habitual diet, fiber content, fermented foods usage and dietary pattern, would help to improve interpretation of results in further researches.

This research had several advantages. Randomized design excluded the influence of selection bias, and baseline demographic and clinical variables were equally distributed among intervention and control groups. The repeated stool testing at baseline, second and fourth weeks enabled researchers to evaluate the temporal changes in microbiota. Usage of 16S rRNA sequencing allowed conducting objective microbiota analysis, whereas fecal SCFA levels helped to estimate its functional metabolism rather than only taxonomic diversity (21). Intervention was realistic and culturally acceptable, involving culinary spices used as part of regular meal, what might make intervention feasible in the case of further success.

A few limitations need to be addressed before publication. The study sample size was 56 participants, where there were four cases of losses to follow-up. This limited the statistical power and generalizability. There were no data on whether the microbial and metabolic changes persisted beyond the period evaluated by the four weeks' follow-up assessment. In addition, there was no placebo used to control for the performance and expectation bias, where the control group maintained their habitual dietary intake. It is possible that uncontrolled dietary intake in terms of fiber, fermented food, spices, and other nutritional factors impacted the results for both the microbiota and SCFAs. The information on the antibiotic type, dosage, indications, and duration of administration were not adequately provided, despite the fact that these parameters greatly impact the severity and the course of antibiotic-induced dysbiosis (22).

Further methodological details of the experiment need to be included. In particular, the authors must report on allocation concealment, blinding procedure, sequencing region and primer set, sequencing platform, minimum read depth, bioinformatic pipeline, taxonomy database, rarefaction/normalization method, GC-MS sample preparation protocol, calibration standards, quality control samples, and missing data handling. The trial registration and ethical approval details need to be reported too. Chao1 richness, Bray-Curtis beta diversity, SCFA subtypes, butyrate values, if they were analyzed or discussed in the

Results, are required. The between-group mean differences, 95% confidence intervals, and effect sizes for all primary and secondary outcomes should be reported (23).

These findings show that the regular use of mixed spices in food is linked to the faster recovery of microbial richness and fecal SCFAs following antibiotic administration among healthy individuals. There is encouraging early evidence about the feasibility of an inexpensive and culturally sensitive strategy for the restoration of the gut microbiota after the use of antibiotics, but the current findings should be considered carefully. Further research with placebo-controlled trials, precise dietary information, class of antibiotics, extended duration of study, and multi-omic analysis is needed.

CONCLUSION

In this randomized controlled trial, daily consumption of a standardized mixture of culinary spices for four weeks following standard antibiotic treatment was linked with a significantly greater short-term increase in gut bacterial diversity and fecal short-chain fatty acid levels and small improvements in GI symptoms when compared to regular diet. The results suggest the feasibility of the use of such dietary approaches in restoring gut microbiota following antibiotic treatment, though more research is needed.

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