Original Article

Effects of Probiotic Supplementation on Treatment Outcomes of Patients with Active Tuberculosis: A Systematic Review of Randomized Controlled Trials

Indry Agatha Rihi Pake,[*] Hafidz Abdullah,[**] Lufthi Fahreza,[***] Jeremiah Suwandi,[****] Zuresh Shafira Sharafina Faisal,[***] Bilqish Karidza,[***] Gilbert Lazarus[*****]

**Faculty of Medicine, Tarumanagara University, Jakarta, Indonesia*
***Faculty of Medicine, Islamic University of Indonesia, Yogyakarta, Indonesia*
****Faculty of Medicine, YARSI University, Jakarta, Indonesia*
*****Faculty of Medicine, Pelita Harapan University, Tangerang, Indonesia*
******Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*

Abstract

Introduction: *Tuberculosis, one of the most globally burdensome infectious diseases, poses high mortality and morbidity risks due to the adverse effects of treatment and disease-related complications, resulting in poor treatment adherence. Despite probiotics, treatment supplements containing live bacteria and having known immunomodulatory effects, their impact on tuberculosis therapy remains understudied. Hence, this systematic review aims to explore the effect of probiotic supplementation on the outcomes of active tuberculosis therapy, including but not limited to its effectiveness in improving tuberculosis treatment and host immunity, as well as its adverse events.*
Method: *A systematic review was performed by searching relevant primary clinical studies from PubMed, Embase, Google Scholar, and Clinicaltrials.gov. The included studies were assessed for risk of bias with the Revised Cochrane risk-of-bias tool for randomized trials, and the findings were synthesized qualitatively.*
Results: *A total of five randomized controlled trials involving 926 patients were included in this systematic review. Our findings revealed that Lactobacillus casei probiotic supplementation significantly reduced the incidence and duration of gastrointestinal side effects during tuberculosis therapy, particularly vomiting, decreased appetite, and constipation. Additionally, probiotics displayed the potential to enhance immunity by increasing lymphocyte cell counts, downregulating proinflammatory cytokines, maintaining immunoglobulin A levels in saliva, mitigating oxidative stress in gastrointestinal organs, and ameliorating gut dysbiosis.*
Conclusion: *These results highlight the advantageous impact of probiotics on active tuberculosis therapy outcomes. Further studies involving a more heterogeneous population investigating the adverse effects following probiotics use, and the long-term effects of probiotics supplementation are required to substantiate our findings.*

Keywords: *Probiotic, Gut microbiota, Tuberculosis, Adverse event, Host immunity*

Korespondensi: **Gilbert Lazarus**
E-mail: *gilbert.lazarus@ui.ac.id*

Pengaruh Suplementasi Probiotik terhadap Luaran Pengobatan Pasien dengan Tuberkulosis Aktif: Sebuah Kajian Sistematis

Indry Agatha Rihi Pake,[*] Hafidz Abdullah,[**] Lufthi Fahreza,[***] Jeremiah Suwandi,[****] Zuresh Shafira Sharafina Faisal,[***] Bilqish Karidza,[***] Gilbert Lazarus[*****]

**Fakultas Kedokteran Universitas Tarumanagara, Jakarta, Indonesia*
***Fakultas Kedokteran Universitas Islam Indonesia, Yogyakarta, Indonesia*
****Fakultas Kedokteran Universitas YARSI, Jakarta, Indonesia*
*****Fakultas Kedokteran Universitas Pelita Harapan, Tangerang, Indonesia*
******Fakultas Kedokteran Universitas Indonesia, Jakarta, Indonesia*

Abstrak

Pendahuluan: *Tuberkulosis merupakan salah satu penyakit infeksi dengan beban mortalitas dan morbiditas tertinggi di dunia, yang diakibatkan oleh tingginya efek samping pengobatan dan komplikasi penyakit yang menyebabkan rendahnya tingkat kepatuhan pengobatan. Meskipun probiotik, suplementasi pengobatan yang mengandung bakteri hidup, diketahui memiliki efek imunomodulator, efek probiotik pada terapi tuberkulosis masih minim diteliti. Oleh karena itu, kajian sistematis ini bertujuan untuk meneliti pengaruh suplementasi probiotik terhadap luaran terapi tuberkulosis aktif, termasuk pengaruhnya dalam meningkatkan efektivitas terapi tuberculosis dan imunitas host, serta efek samping konsumsi probiotik.*
Metode: *Sebuah kajian sistematis dilakukan dengan menjadi studi klinis primer yang relevan di PubMed, Embase, Google Scholar, dan Clinicaltrials.gov. Studi-studi yang memenuhi kriteria inklusi dinilai risiko biasnya dengan the Revised Cochrane risk-of-bias tool for randomized trials, dan temuan dari studi-studi tersebut kemudian disintesis secara kualitatif.*
Hasil: *Lima uji klinis acak dengan total 926 pasien dimasukkan ke dalam penelitian ini. Dua studi melaporkan bahwa suplementasi probiotik dengan Lactobacillus casei secara signifikan menurunkan kejadian dan durasi efek samping gastrointestinal terapi tuberkulosis khususnya vomitus, penurunan nafsu makan, dan konstipasi. Selain itu, empat studi menunjukkan bahwa probiotik dapat meningkatkan imunitas tubuh dengan meningkatkan jumlah sel limfosit, menurunkan jumlah sitokin proinflamatori, mencegah penurunan kadar imunoglobulin A di saliva, menurunkan stres oksidatif di organ gastrointestinal, serta memperbaiki disbiosis usus.*
Kesimpulan: *Probiotik terbukti memiliki efek menguntungkan terhadap luaran terapi tuberkulosis aktif. Studi-studi lebih lanjut dengan populasi yang lebih heterogen dan meneliti efek samping serta efek jangka panjang penggunaan probiotik dibutuhkan untuk membuktikan temuan studi ini.*

Kata kunci: *Probiotik, Mikrobiota usus, Tuberkulosis, Efek samping, Imunitas*

Introduction

Tuberculosis, a chronic lung disease caused by *Mycobacterium tuberculosis*, continues to be a significant global health challenge, with 1.25 million deaths in 2023.[1] It is estimated that about one-quarter of the world's population is infected with *M. tuberculosis*, and about 1 in 10 of these individuals are vulnerable to developing active tuberculosis during their lifetime.[2] Based on data from the 2024 Tuberculosis Global Report, Indonesia ranks second globally after India, with an estimated 1,090,000 tuberculosis cases.[1]

The elevated mortality burden of tuberculosis can largely be ascribed to suboptimal treatment compliance stemming from the pronounced side effects associated with tuberculosis medications. These side effects frequently result in inadequate adherence to the prescribed treatment regimen, thus potentially giving rise to drug-resistant *M. tuberculosis* strains.[3] Additionally, a significant concern revolves around the attenuation of patients' immune responses upon tuberculosis infection, which may lead to disease exacerbation and the manifestation of diverse tuberculosis-related complications.[4] These factors underscore the need to explore innovative, more effective strategies to enhance conventional tuberculosis treatment.[5]

In recent years, there has been grow-

ing interest in exploring natural and biological products as potential novel remedies for active tuberculosis infection. Among these, probiotics have emerged as potential candidates for immune system modulation. The administration of probiotics has demonstrated the potential to enhance the activity of alveolar macrophages and natural killer (NK) cells, thus leading to increased cytokines production as the basis of immune response pathways in suppressing tuberculosis.[6] Nevertheless, the use of probiotics in active tuberculosis patients remains a subject of ongoing investigation.[2] This systematic review aims to analyze the effects of probiotics supplementation in improving the treatment outcomes of patients with active tuberculosis, thus diminishing the devastating disease burden caused by tuberculosis.

Method

This systematic review followed the Cochrane Handbook for Systematic Reviews of Intervention v6.4[7] and was reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2020 statement.[8]

Search Strategy

The literature search was performed by screening peer-reviewed (PubMed) and grey-literature databases (EMBASE, Google Scholar, Clinicaltrials.gov), searching for primary clinical studies conducted on human participants, including but not limited to case series, cross-sectional, case control, cohort, and experimental studies; evaluating the effects of probiotics supplementation in patients receiving active tuberculosis treatments, using the keywords and similar terms of probiotics and tuberculosis (Table 1). We selected the literature databases following the recommendation by Bramer et al., where the combinations of EMBASE, PubMed (MEDLINE), and Google Scholar yielded an overall recall of 95.9%.[9] We were unable to perform literature search in Web of Science due to lack of access. Additionally, we added Clinicaltrials.gov to our search as the database contains records of both published and unpublished clinical trials, which we believe would be invaluable to our review. The exclusion criteria were: (a) case reports, editorials, reviews, or conference proceedings, (b) studies involving latent tuberculosis infection, (c) irretrievable full-text articles, or (d) articles not in English of Bahasa Indonesia. The search was conducted independently by two investigators (IA and JS, ZS and BK), and any discrepancies were resolved by the independent assessment of a third reviewer (GL).

Table 1. Details on Keywords Used to Search for Relevant Literature

No	Database	Keyword	Hits
1	PubMed	((“probiotics”[MeSH Terms]) OR (“yogurt”[MeSH Terms]) OR (“lactobacillus”[MeSH Terms]) OR (“streptococcus thermophilus”[MeSH Terms]) OR (“lactococcus”[MeSH Terms]) OR (“bifidobacterium”[MeSH Terms]) OR (“saccharomyces”[MeSH Terms]) OR yoghurt OR yogurt OR yoghourt OR probiot*) AND ((“tuberculosis”[MeSH Terms]) OR “wasting disease” OR “white plague” OR TB)	178
2	EMBASE	#1 ‘tuberculosis’/exp #2 TB #3 ‘probiotic agent’/exp OR ‘yoghurt’/exp OR ‘lactobacillus’/exp OR ‘lactococcus’/exp OR ‘bifidobacterium’/exp OR ‘saccharomyces’/exp OR ‘streptococcus thermophilus’/exp #4 probiot* OR yoghurt OR yoghourt #5 #1 OR #2 #6 #3 OR #4 #7 #5 AND #6	576
3	Google Scholar	(“probiotics” OR “yogurt” OR “yoghurt” OR “lactobacillus” OR “lactococcus” OR “saccharomyces” OR “bifidobacterium” OR “streptococcus thermophilus”) AND “tuberculosis”	40
4	Clinicaltrials.gov	Conditions or disease: Tuberculosis Intervention: Probiotics	2

Data Extraction and Risk of Bias Assessment

The subsequent data was extracted from each study: (a) the last name of the first contributor and year of publication; (b) study characteristics including recruitment period, study design, geographical location,

population, and details on intervention and comparators; (c) participant demographics, comprising of sample sizes, age, and sex; and (d) outcomes of probiotics supplementation on tuberculosis treatment. The outcomes considered for this systematic review relate to the clinical effect of probiotics supplementation, i.e., improvement in tuberculosis disease including but not limited to symptom and disease status, adverse effects of anti-tuberculosis drugs and treatment compliance, and lung function and host immunity status.

The included studies were further assessed for risk of bias using the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2.0), with the overall risk of bias categorized as low, moderate, or high.[10] Data extraction and risk of bias assessments were conducted by a single reviewer (IA, JS, ZS, BK), and a second investigator (GL) independently checked the accuracy of the extracted data and the quality assessment.

Data Analysis

Data were presented as frequencies and proportions (%) for dichotomous variables, and as mean ± standard deviation (SD) or median and interquartile range (IQR) for continuous variables. Due to substantial heterogeneity in the reported outcomes, and types and dosages of probiotic supplementation, we refrained from performing a quantitative meta-analysis.[11] Instead, we synthesized the available evidence qualitatively and narratively, highlighting on the relevant and significant findings.

Results

Study Search and Characteristics

Out of 754 articles identified through the database search, 71 were deduplicated, and 646 were excluded following title and abstract screening, resulting in 37 full-text articles to be reviewed. Of these, 22 were excluded due to inappropriate study design, six due to incompatible language, and two did not involve probiotics supplementation, and one due to no available full-text (conference abstract) (Figure 1). Consequently, this systematic review included five randomized controlled trials (RCTs) cumulating a total of 926 patients (one double-blind and four open-label).[12–15] These trials were conducted between 2016 and 2021, and three were conducted in China[13,14,16] and two in Indonesia.[12,15] All studies investigated the effects of probiotics supplementation on the treatment of active pulmonary tuberculosis. Three studies investigated the effect of Lactobacillus casei supplementation,[13,14,16] one investigated the effect of *L. acidophilus* and *Bifidobacterium longum,*[12] and one did not specify the composition of probiotics.[15] Of the 926 included patients, most were male (54.5%, 505/926 patients) with a mean age of 34.6±17.3 years (Table 2). Risk of bias assessments revealed moderate risk for three studies,[12,14,16] high risk for one study,[15] and low risk for one study[13] (Figure 2). The identified potential bias arose from possible selection,[14–16] blinding,[16] and attrition bias[12,15] among the included studies.

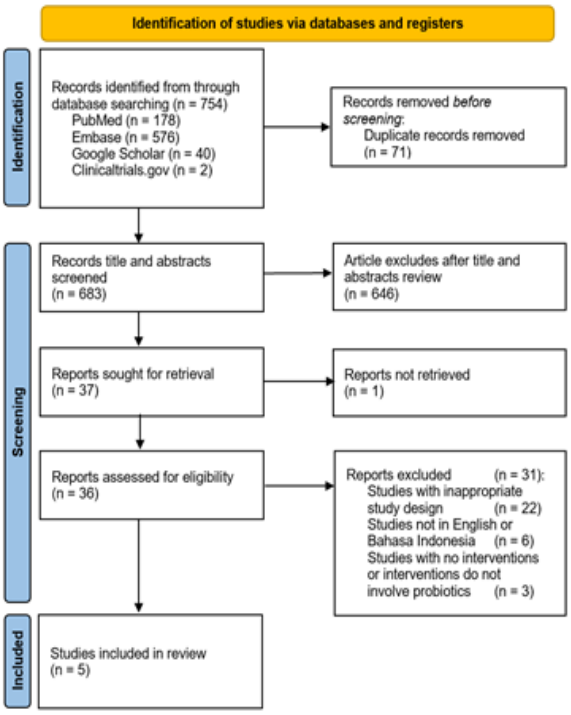

Figure 1. PRISMA Diagram Flow

Two articles investigated the effects of probiotics supplementation on the occurrence and duration of anti-tuberculosis drug-associated gastrointestinal adverse events,[13,14] and four investigated the effects of probiotics supplementation on host immunity, particularly on gut microflora,[12,14] salivary secretory immunoglobulin A (sIgA) secretion[12] and inflammatory markers.[15,16]

Outcomes

Effect of Probiotic Supplementation on Anti-tuberculosis Drugs Adverse Effects

Two studies reported that probiotic supplementation may prevent and alleviate

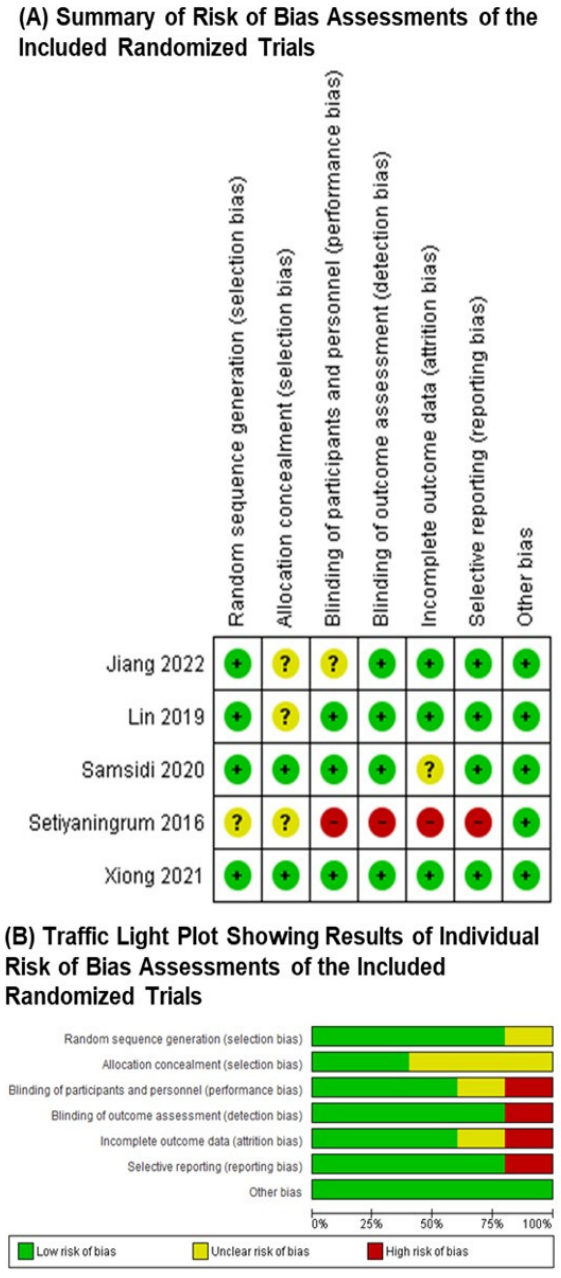

Figure 2. Results of (A) Summary-Level and (B) Individual-Level Risk of Bias Assessments of the Included Randomized Controlled Trials Assessed with the Revised Cochrane Risk-of-Bias Tool for Randomized Trials (RoB2.0)

adverse effects following the administration of anti-tuberculosis drugs, as shown by the reduction in the occurrence and duration of drug-related adverse events following the administration of tuberculosis treatments.[13,14] Lin et al. showed that both low and high doses of *L. casei* supplementation reduced the occurrence and the duration of total gastrointestinal adverse events (29.4% and 37.6% vs 50.0%, p=0.001; median 0 days [interquartile range (IQR) 0-135] and 0 days [0-40] vs 0.5 days [0-180], p=0.005), particularly vomiting (0.0% and 1.6% vs 4.3%, p=0.011; median 0 days [IQR0-30] and 0 days [0-0] vs 0 days [0-3], p=0.037) appetite loss (3.8% and 7.9% vs 13.8%, p=0.003; median 0 days [IQR0-60] and 0 (0-30) vs 0 (0-20), p=0.013), and constipation (incidence only for low dose: 15.1% vs 26.1%, p=0.028; duration: median 0 days (0-60) and 0 (0-20) vs 0 (0-60), p=0.040). Similarly, Xiong et al. also mentioned that a low dose (1x1010 CFU) of *L. casei* supplementation reduced the incidence of elevated total bilirubin levels (1.2% vs 9.7%, p=0.013). In comparison, high-dose (2x1010 CFU) supplementation reduced the incidence of elevated alkaline phosphatase enzyme (0.0% vs 4.9%, p=0.024). However, it did not reduce the incidence of liver injury during the intensive phase of tuberculosis treatment nor affected the levels of transaminase and gamma-glutamyl transpeptidase enzymes (Table 3). In addition, the reduction of the incidence of vomiting and appetite loss was observed in a dose-response manner.

Effect of Probiotic Supplementation on Host Immunity and gut microbiota

Three studies investigated the effect of probiotic supplementation on host immunity and gut microbiota.[12,13,16] Probiotic supplementation for over four weeks, coupled with zinc, significantly increased lymphocyte count (mean difference [MD] 0.21± standard deviation 0.35, p=0.002) and decreased neutrophil-to-lymphocyte (-0.20±2.40, p=0.008) and monocyte count (-0.12±0.37, p=0.026). These levels were considerably higher than the control group (partial eta squared 0.128, p=0.010).[15] However, in another study by Jiang, et al[16] *L. casei* supplementation did not improve white blood cell counts.[16] Nonetheless, the study reported that high-dose *L. casei* supplementation (2x1010 CFU) for four weeks, not low-dose (1x1010 CFU), may reduce proinflammatory cytokines such as tumor necrosis factor-α (0.60±0.36 vs 0.91±0.36 pg/mL, p=0.012), interleukin-6 (0.11±0.48 vs 0.51±0.58, p=0.057), interleukin-10 (0.42±0.54 vs 0.91±0.36, p=0.001), and interleukin-12 (0.72±0.29 vs 1.04±0.22, p<0.001). In addition, synbiotic supplementation with *L. acidophilus* and *B. longum* for one month also prevented the significant decline in salivary sIgA titers (MD 7.25 µg/mL, p>0.05), unlike the control group (-52.48 µg/mL, p<0.05)15. High dose *L. casei* supplementation (2x1010 CFU) also reduced plasma concentration

Table 2. Characteristics of the Included Studies and Participants

Author (Year)	Study Characteristics				Subject Characteristics							
					Intervention				Control			
	Recruitment Period	Study Design	Location	Population	Details	N	Mean Age (years)	Male Sex (%)	Details	N	Mean Age (years)	Male Sex (%)
Jiang (2022)16	Dec 2017 – Jan 2019	RCT, open-label	China	Pulmonary TB	Anti-TB drug + 1x1010 CFU L. casei	16	26.1±10.8	11 (68.8)	Anti-TB drug only	15	33.5±15.2	8 (53.3)
					Anti-TB drug + 2x1010 CFU L. casei	16	26.1±10.6	8 (50.0)				
Xiong (2021)14	Dec 2017 – Jan 2019	RCT, open-label	China	Pulmonary TB	Anti-TB drug + 1x1010 CFU L. casei	106	31.0±23.3	61 (57.5)	Anti-TB drug only	105	31±25	70 (66.7)
					Anti-TB drug + 2x1010 CFU L. casei	114	29.0±24.0	58 (50.9)				
Samsidi (2020)12	Nov 2009 - Aug 2010	RCT, double-blind	Indonesia	Pulmonary TB	Anti-TB drug + milk-based protein supplement + synbiotic (4x1017 L. acidophilus and 3x102 B. longum) + micronutrient	43	NR	NR	Anti-TB drug + milk-based protein supplement	38	NR	NR
Lin (2019)13	Dec 2017 – Jan 2019	RCT, open-label	China	Pulmonary TB	Anti-TB drug + 1x1010 CFU L. casei	132	37.2 ± 14.5	79 (59.8)	Anti-TB drug only	144	39.2 ± 16.5	93 (64.6)
					Anti-TB drug + 2x1010 CFU L. casei	143	36.2 ± 15.1	81 (56.6)				
Setiyaningrum (2016)15	NR	RCT, open-label	Indonesia	Pulmonary TB	Anti-TB drug + probiotics and zinc a day for four weeks	27	43.2±14.2	21 (77.8)	Anti-TB drug only	27	41.56+11.70	15 (55.6)

B. longum, Bifidobacterium longum; CFU, colony forming unit; L. acidophilus, Lactobacillus acidophilus; L. casei, Lactobacillus casei; NR, not reported; RCT, randomized controlled trial; TB, tuberculosis.

of fatty acid binding protein (median 1.14 [IQR1.63] vs 3.13 [3.40], p=0.002), zonula occludens-1 (MD -33.55, p=0.001), and lipopolysaccharide (MD -33.42, p=0.020) compared to control.

In terms of gut microflora, supplementation with *L. acidophilus* and *B. longum* for one month significantly reduced Lactobacillus sp. (9.85 vs 9.76 log-CFU/g, p<0.05) and Bifidobacterium sp. (5.93 vs 5.35 log-CFU/g, p<0.05) colonies in feces. In contrast, supplementation with *L. casei* decreased Bacteroides (p<0.001) and increased *Actinobacteria* (p<0.001) and Firmicutes colonies (only for the high-dose group, p=0.003). Of the nine bacterial genes, seven showed a significant

Table 3. Outcomes Following Probiotics Supplementation in Patients Receiving Active Tuberculosis Treatments

Author (Year)	Outcomes
Jiang (2022)[16]	● **High dose *L. casei* supplementation (2x1010 CFU) resulted in a significantly lower concentrations of tumor necrosis factor-α (0.60±0.36 pg/mL vs 0.88±0.18 in low-dose supplementation and 0.91±0.36 in control group; p=0.012), interleukin-6 (0.11±0.48 vs 0.53±0.46 and 0.51±0.58; p=0.057), interleukin-10 (0.42±0.54 vs 0.98±0.21 and 0.91±0.36; p=0.001), and interleukin-12 (0.72±0.29 vs 1.07±0.10 and 1.04±0.22; p<0.001)** ● Compared to low-dose supplementation of *L. casei* (1x1010 CFU) and control, high-dose *L. casei* supplementation (2x1010 CFU) significantly upregulated plasma metabolites of phosphatidylserine, maresin 1, phosphatidylcholine, L-saccharopine, and pyridoxamine; and downregulated N-acetylmethionine, L-tryptophan, phosphatidylethanolamine, and phenylalanine; which were strongly correlated with inflammatory cytokines. ● *L. casei* supplementation, either with high (2x1010 CFU) or low dose (1x1010 CFU), did not affect the number of white blood cells (p=0.580), neutrophils (p=0.733), lymphocytes (p=0.210), monocytes (p=0.798), eosinophils (p=0.113), and interferon-γ (p=0.912)
Xiong (2021)[14]	● *L. casei* supplementation, either with high (2x1010 CFU) or low dose (1x1010 CFU), did not reduce the incidence of liver injury during the intensive phase of tuberculosis treatment compared to control (3.5% and 4.7% versus 5.7%, p=0.738). ● ***L. casei* supplementation, both with high (2x1010 CFU) and low dose (1x1010 CFU), reduced the incidence of elevated ALP enzyme compared to control (0.0% vs 4.9%, p=0.024; and 3.5% vs 4.9%, p=0.092).** ● ***L. casei* supplementation, both with high (2x1010 CFU) and low dose (1x1010 CFU), reduced the incidence of elevated total bilirubin level (3.9% vs 9.7%, p=0.101; 1.2% vs 9.7%, p=0.013).** ● No significant difference in the incidence of elevated ALT, AST, and GGT enzymes among the groups. ● **There was significantly lower plasma concentration of fatty acid binding protein (1.14 [1.63] vs 3.13 [3.40], p=0.002), zonula occludens-1 (93.92±37.71 vs 127.47±47.68, p=0.001), and lipopolysaccharide (75.43±33.34 vs 108.85±66.11, p=0.020) between those with a high dose of *L. casei* supplementation (2x1010 CFU) and control.** ● **Compared to control, high (2x1010 CFU) and low dose (1x1010 CFU) of *L. casei* supplementation showed a decrease of Bacteroidetes (both p<0.001) and an increase of Actinobacteria (both p<0.001) and Firmicutes (p=0.089 and p=0.003).**
Samsidi (2020)[12]	● **Supplementation with a milk-based protein supplement and synbiotic containing 17.6 log-CFU Lactobacillus acidophilus and 2.5 log-CFU Bifidobacterium longum for one month significantly reduced *Lactobacillus sp*. and *Bifidobacterium sp.* colonies in feces compared to control (9.85 vs 9.76 log-CFU/g, p<0.05; and 5.93 vs 5.35 log-CFU/g, p<0.05).** ● **Synbiotic supplementation significantly reduced *Lactobacillus sp.* colonies in feces after six months (5.43±1.71 vs 6.34±2.58 log-CFU/g, [MD -0.91 log-CFU/g, p<0.001]), and increased *Bifidobacterium sp.* colonies after 1 and 2 months (9.81±0.60 vs 9.53±0.84 log-CFU/g [MD 0.28 log-CFU/g, p<0.05]; 9.87±0.40 vs 9.53±0.84 log-CFU/g [MD 0.34 log-CFU/g, p<0.05]),** but not after six months (9.73±0.72 vs 9.53±0.84 log-CFU/g [MD 0.20 log-CFU/g, p>0.05]). ● Salivary secretory IgA titers in the treatment group did not change significantly over the treatment period (1 month vs baseline: 307.25±289.85 vs 300.00±300.00 µg/mL, [MD 7.25 µg/mL, p>0.05]; 3 months vs baseline: 269.57±333.33 vs 300.00±300.00 µg/mL [MD -30.43 µg/mL, p>0.05]; 6 months vs baseline: 256.52±346.38 vs 300.00±300.00 µg/mL [-43.48 µg/mL, p>0.05]), unlike the control group which secretory IgA titers decreased significantly after six months (174.47±425.53 vs 226.95±373.05 [MD -52.48, p<0.05]).
Lin (2019)[13]	● *L. casei* supplementation, both low (1x1010 CFU) and high dose (2x1010 CFU), reduced the incidence of anti-tuberculosis drug-associated gastrointestinal adverse events compared to control (anti-tuberculosis drugs only): ○ **Total gastrointestinal adverse events: 37/126 (29.4%) and 50/133 (37.6%) vs 69/138 (50.0%), p=0.034 (p=0.001 between low dose and control, p=0.040 between high dose and control)** ○ Nausea: 12/126 (9.5%) and 17/133 (12.8%) vs 17/138 (12.3%), p=0.913 ○ **Vomiting: 2/126 (1.6%) and 0/133 (0.0%) vs 6/138 (4.3%), p=0.011 (p=0.030 between high dose and control)** ○ Diarrhea: 5/126 (4.0%) and 7/133 (5.3%) vs 7/138 (5.1%), p=0.946 ○ Flatulence: 2/126 (1.6%) and 3/133 (2.3%) vs 2/138 (1.4%), p=0.616 ○ **Appetite loss: 10/126 (7.9%) and 5/133 (3.8%) vs 19/138 (13.8%), p=0.003 (p=0.004 between high dose and control)** ○ **Constipation: 19/126 (15.1%) and 27/133 (20.3%) vs 36/138 (26.1%), p=0.232 (p=0.028 between low dose and control)** ● *L. casei* supplementation, both low (2x1010 CFU) and high dose (1x1010 CFU), shortened the duration of anti-tuberculosis drug-associated gastrointestinal adverse events compared to control (anti-tuberculosis drugs only): ○ **Total gastrointestinal adverse events: median 0 days (IQR0-40) and 0 (0-135) vs 0.5 (0-180), p=0.005** ○ Nausea: median 0 days (IQR0-60) and 0 (0-30) vs 0 (0-20), p=0.762 ○ **Vomiting: median 0 days (IQR0-30) and 0 (0-0) vs 0 (0-3), p=0.037** ○ Diarrhea: median 0 days (IQR0-15) and 0 (0-10) vs 0 (0-6), p=0.899 ○ Flatulence: median 0 days (IQR0-15) and 0 (0-30) vs 0 (0-60), p=0.871 ○ **Appetite loss: median 0 days (IQR0-60) and 0 (0-20) vs 0 (0-20), p=0.013** ○ **Constipation: median 0 days (IQR0-60) and 0 (0-20) vs 0 (0-60), p=0.040**
Setiyaningrum (2016)[15]	● **Combination supplementation with probiotics and zinc for over four weeks significantly increased lymphocyte count (0.21±0.35, p=0.002) and decreased NLR (-0.20±2.40, p=0.008) and monocyte count (-0.12±0.37, p=0.026)** ● Compared to control, combination supplementation with probiotics and zinc for over four weeks significantly increased lymphocyte count (0.21±0.35 vs -0.06±0.41, p=0.013) and decreased monocyte count (-0.12±0.37 vs -0.03±0.03, p=0.040), but not for NLR (-0.20±2.40 vs -0.72±2.26, p=0.239) ● After four weeks, **the lymphocyte counts were higher in patients receiving combination supplementation with probiotics and zinc compared to control (2.21±0.86 vs 1.89±0.61, p=0.010, partial eta squared=0.128),** but not for NLR (3.73±3.03 vs 3.41±2.22, p=0.40, partial eta squared=0.014) and monocyte count (0.84±0.40 vs 0.80±0.37, p=0.46, partial eta squared=0.011)

increase in abundance in the low-dose group compared with the control group (*Bifidobacterium, Ruminococcus_torques group, Collinsella, Mitsuokella, Blautia, Streptococcus* and *Enterococcus*). Seven bacterial genera were identified as biomarkers that differentiated the high-dose group from the control group. Among these seven bacterial genes, five showed a significant increase in abundance compared to the control (*Bifidobacterium, Streptococcus, Blautia, Erysipelotrichaceae_UCG003* and *Collinsella*), while two showed a significant decrease in relative abundance (*Lachnoclostridium* and *Bacteroides*).

Discussion

In recent years, the management of tuberculosis has become increasingly challenging, primarily due to the significant adverse effects associated with tuberculosis medications. These adverse effects have contributed to low patient compliance, high rates of loss to follow-up, and the emergence of drug-resistant strains. Consequently, there is a pressing need for innovative strategies to enhance treatment adherence among patients receiving active tuberculosis therapy. This systematic review highlights the potential benefits of probiotic supplementation in mitigating the incidence, occurrence, and duration of adverse events associated with anti-tuberculosis drugs, as well as its ability to bolster the patient's immune system and alleviate gut dysbiosis induced by tuberculosis treatment.

Patients undergoing anti-tuberculosis drug therapy commonly encounter a spectrum of adverse events. Isoniazid is known to induce peripheral neuropathy, skin rashes, hepatitis, and lethargy, while rifampicin may lead to gastrointestinal discomfort, including abdominal pain, nausea, and vomiting. Pyrazinamide can cause joint pain and hepatotoxicity, ethambutol may result in retrobulbar neuritis, and streptomycin often triggers vestibular and auditory toxicity, along with kidney damage.[17] Prior reviews have estimated that adverse events occur in approximately 8-85% of patients receiving first-line anti-tuberculosis drugs, with the majority manifesting during the intensive phase. Patients receiving second-line anti-tuberculosis drugs face even higher risks, with adverse events documented in about 69-96% of cases. Among these, gastrointestinal symptoms are prevalent, ranging from mild symptoms such as nausea, vomiting, flatulence, appetite loss, and diarrhea/constipation to more severe complications like gastrointestinal bleeding and fulminant hepatitis.[18] Notably, a study conducted in Jakarta reported that 73.8% of participants (127 patients) experienced minor adverse reactions. In comparison, 26.2% suffered major adverse reactions, with gastrointestinal disorders and drug-induced hepatitis being the most common minor and major adverse events, respectively (34% and 60%).[19] Left untreated, these adverse events significantly impact patients' quality of life, leading to poor treatment adherence and potential treatment failure, as supported by Setiawan et al.'s study, which found that patients experiencing adverse events are ten times more likely to default from tuberculosis treatment.[19] This study reveals that probiotic supplementation with *L. casei* can reduce the incidence and duration of gastrointestinal adverse events resulting from anti-tuberculosis drug administration, particularly vomiting, appetite loss, and constipation. This aligns with previous studies suggesting that *L. casei* may alleviate gastrointestinal discomfort induced by stress.[20] The beneficial effects of probiotics on alleviating gastrointestinal symptoms are thought to be mediated through alterations in the gut microbiome and metabolome. Probiotics containing *Lactobacillus* and *Bifidobacterium* may promote the growth of bile salt hydrolase-producing bacteria, capable of deconjugating bile acids, ultimately leading to reduced free bile acid levels and a consequent reduction in nausea and vomiting symptoms.[21] In addition, probiotics may downregulate inflammatory cytokines and modulate serotonin, which stimulates neuropeptide Y and appetite-regulating hormones, thus improving the appetite of the affected patients.[22] Lastly, probiotics may also regulate fecal microbiota and increase organic acid levels in the gut, thus promoting intestinal peristalsis and shortening colon transit time, consequently alleviating symptoms of constipation.[23]

In the human digestive system, commensal microbiota not only colonize the host's intestinal tract but also engage in symbiotic interactions that play a vital role in maintaining the host's health and influencing disease outcomes.[24] Disturbances in the balance of gut microbiota, often induced by antimicrobial drugs, including anti-tuberculosis antibiotics, can harm gut immunity and lead to adverse gastrointestinal symptoms.[25] This review reveals that probiotics may bolster host immunity and promote a healthier gut microbial balance. This is evident in the increased counts of differential white blood cells and neutrophil-to-lymphocyte ratio (NLR), re-

duced levels of reactive oxygen species and proinflammatory cytokines, as indicated by lower cholestasis-related liver markers and plasma lipopolysaccharide, improved intestinal permeability, optimized gut microbiota composition, and sustained secretory IgA levels in saliva. Additionally, probiotic supplementation has been shown to positively influence the levels of IFN-γ and IL-12 in the early phases of tuberculosis treatment, facilitating the eradication of M. tuberculosis bacteria, particularly during the intensive treatment phase.[2] A randomized controlled trial by Horvath et al. demonstrated a significant increase in neutrophil resting burst and serum killing capacity in patients who received probiotics for six months, compared to the placebo group.[26] These findings suggest that probiotics can enhance host immunity and mitigate gut dysbiosis induced by tuberculosis treatment. Thus, they highlight the potential of probiotics as a complementary approach to tuberculosis treatment, with the potential to improve patient outcomes and treatment adherence.[12–15]

Probiotic supplementation also plays a pivotal role in eradicating *M. tuberculosis* from infected hosts. In an in-vivo study by Youn, et al., it was observed that Lactobacillus supplementation significantly increased IgA levels and reduced IL-6 and TNF-α in the lungs of infected mice, contributing to improved survival rates.[27] Moreover, probiotics can reduce TNF-α and IL-1β levels in bronchoalveolar lavage fluid, indicating reduced lung inflammation.[28] Additionally, probiotics may activate granulocyte macrophage-colony stimulating factor (GM-CSF), which can aid in pathogen elimination through mechanisms like phagocytosis, reduced reactive oxygen species production, and activation of the extracellular signal-regulated kinase (ERK) signalling pathway. This ERK pathway is crucial in regulating granuloma inflammation and M. tuberculosis growth. Consequently, macrophages release IL-1β, which possesses antimicrobial properties, further promoting bacterial eradication. These findings proved the important role of probiotics in maintaining a healthy gut-lung axis, especially as research has found that enhancing the gut microbiota's composition may help mitigate the severity of respiratory illnesses, including active pulmonary tuberculosis.[29]

In recent years, there has been a significant surge in the use of probiotics as adjuncts to conventional drug therapies.[30] Research on probiotics has seen a 34-fold increase since 1998, although very few were translated into clinical recommendations.[31] While these trends indicate a favorable inclination toward the use of probiotics, it is essential to acknowledge that a majority of this research has been conducted in developed countries. In contrast, data from developing countries, particularly those heavily burdened by tuberculosis, such as India, Indonesia, and China, remains limited. Several barriers to the implementation of probiotic supplementation in developing nations exist, including shifting demographic profiles, higher disposable incomes, and sedentary lifestyles that may place probiotics lower on the healthcare priority list for these populations. Nevertheless, the increasing health literacy among global communities, combined with the high tolerability of probiotic supplements and minimal reports of side effects, suggests the potential for probiotics to serve as beneficial health supplements for both healthy individuals and those with various health conditions.[30,32]

This review encountered several limitations. The relatively small sample sizes in the included studies predominantly resulted in a moderate-to-high risk of bias, and the heterogeneity of outcomes precluded formal quantitative synthesis through meta-analysis. Furthermore, of the four studies incorporated in this review, two were conducted in China and two in Indonesia. While these two countries rank among those with the highest tuberculosis burdens (2nd and 3rd, respectively),[33] the relatively homogenous study populations limit the generalizability of our findings. The fact that this systematic review only included studies published in English or Bahasa Indonesia also poses potential language bias, although the number of studies excluded due to these language barriers were relatively small. Consequently, further research encompassing larger and higher quality studies involving more diverse populations is imperative to validate our conclusions. These future investigations should also aim to discern the direct effects of probiotic supplementation on adherence to active tuberculosis treatment and its impact on treatment outcomes. Nevertheless, despite these limitations, our findings shed light on the potential beneficial effects of probiotic supplementation in mitigating gastrointestinal-related adverse events and enhancing both host immunity and gut microbiota. All in all, the current available evidence supports the use of probiotics supplementation during active tuberculosis treatment, and has been proven to be safe and potentially effective in enhancing treatment outcomes.

Conclusion

In conclusion, the present systematic review adds to the body of evidence supporting the use of probiotics supplementation in treating active pulmonary tuberculosis. Probiotic supplementation may reduce the incidence and the duration of drug-associated adverse events, enhance host immunity and balance gut microbiota. Further studies involving a more heterogeneous population investigating the adverse effects following probiotics use, and the long-term effects of probiotics supplementation are required to substantiate our findings.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgement

None applicable.

References

1. World Health Organization. Global tuberculosis report. 2024 Nov.
2. Suprapti B, Suharjono, Raising R, Yulistiani, Izzah Z, Nilamsari WP, et al. Effects of probiotics and Vitamin B supplementation on IFN-γ and IL-12 levels during intensive phase treatment of tuberculosis. Indonesian Journal of Pharmacy. 2018;29(2):80–5.
3. Tesfahuneygn G, Medhin G, Legesse M. Adherence to Anti-tuberculosis treatment and treatment outcomes among tuberculosis patients in Alamata District, northeast Ethiopia. BMC Res Notes. 2015 Sep 29;8(1).
4. Luies L, Preez I du. The echo of pulmonary tuberculosis: Mechanisms of clinical symptoms and other disease-induced systemic complications. Vol. 33, Clinical Microbiology Reviews. American Society for Microbiology; 2020. p. 1–19.
5. Arrigoni R, Ballini A, Topi S, Bottalico L, Jirillo E, Santacroce L. Antibiotic Resistance to Mycobacterium tuberculosis and Potential Use of Natural and Biological Products as Alternative Anti-Mycobacterial Agents. Vol. 11, Antibiotics. MDPI; 2022.
6. Mortaz E, Adcock IM, Folkerts G, Barnes PJ, Paul Vos A, Garssen J. Probiotics in the Management of Lung Diseases. Mediators Inflamm. 2013;2013:1–10.
7. Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M. Cochrane Handbook for Systematic Reviews of Interventions version 6.4 (updated August 2023). Welch V, editor. Cochrane; 2023.
8. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Vol. 372, The BMJ. BMJ Publishing Group; 2021.
9. Bramer WM, Rethlefsen ML, Kleijnen J, Franco OH. Optimal database combinations for literature searches in systematic reviews: a prospective exploratory study. Syst Rev. 2017 Dec 6;6(1):245.
10. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898.
11. Deeks JJ, Higgins JPT, Altman DG. Analysing data and undertaking meta-analyses. In: Cochrane Handbook for Systematic Reviews of Interventions. 2019. p. 241–84.
12. Samsidi S, Hardinsyah H, Kusharto CM, Sulaeman A, Alisjahbana B, Ulaganathan V. Synbiotic and nutrients supplement improved of Secretory Immunoglobulin A (sIgA) in treated pulmonary tuberculosis patients. Ann Trop Med Public Health [Internet]. 2020;23(8):1283–91. Available from: https://www.embase.com/search/results?subaction=viewrecord&id=L632243958&from=export
13. Xiong K, Cai J, Liu P, Wang J, Zhao S, Xu L, et al. Lactobacillus casei Alleviated the Abnormal Increase of Cholestasis-Related Liver Indices During Tuberculosis Treatment: A Post Hoc Analysis of Randomized Controlled Trial. Mol Nutr Food Res. 2021 Aug;65(16):e2100108.
14. Lin S, Zhao S, Liu J, Zhang J, Zhang C, Hao H, et al. Efficacy of proprietary Lactobacillus casei for anti-tuberculosis associated gastrointestinal adverse reactions in adult patients: a randomized, open-label, dose-response trial. Food Funct. 2020 Jan;11(1):370–7.
15. Setiyaningrum Z, Darmono SS, Sofro MAU, Dharmana E, Widyastiti NS. Effect of combined probiotics and zinc supplementation on immune status of pulmonary tuberculosis patients. Pakistan Journal of Nutrition [Internet]. 2016;15(7):680–5. Available from: https://www.embase.com/search/results?subaction=viewre-

cord&id=L611765944&from=export
16. Jiang L, Wang J, Xu L, Cai J, Zhao S, Ma A. Lactobacillus casei modulates inflammatory cytokines and metabolites during tuberculosis treatment: A post hoc randomized controlled trial. Asia Pac J Clin Nutr. 2022 Mar;31(1):66–77.
17. Zaleskis R. Postgraduate Course ERS Copenhagen 2005 The side-effects of TB therapy . Breathe. 2005;2(1).
18. Prasad R, Singh A, Gupta N. Adverse drug reactions in tuberculosis and management. Indian Journal of Tuberculosis. 2019 Oct;66(4):520–32.
19. Setiawan S, Ascobat P. Adverse Reactions to First-Line Anti-Tuberculosis Drugs As A Risk Factor Of Pulmonary Tuberculosis Treatment Default In Jakarta, Indonesia. International Journal of Applied Pharmaceutics. 2019 Apr 11;80–3.
20. Li Y, Zhao L, Hou M, Gao T, Sun J, Luo H, et al. Lactobacillus casei Improve Anti-Tuberculosis Drugs-Induced Intestinal Adverse Reactions in Rat by Modulating Gut Microbiota and Short-Chain Fatty Acids. Nutrients. 2022 Apr 17;14(8):1668.
21. Liu AT, Chen S, Jena PK, Sheng L, Hu Y, Wan YJY. Probiotics Improve Gastrointestinal Function and Life Quality in Pregnancy. Nutrients. 2021 Nov 3;13(11):3931.
22. Kazemi A, Noorbala AA, Djafarian K. Effect of probiotic and prebiotic versus placebo on appetite in patients with major depressive disorder: post hoc analysis of a randomised clinical trial. Journal of Human Nutrition and Dietetics. 2020 Feb 23;33(1):56–65.
23. He Y, Zhu L, Chen J, Tang X, Pan M, Yuan W, et al. Efficacy of Probiotic Compounds in Relieving Constipation and Their Colonization in Gut Microbiota. Molecules. 2022 Jan 20;27(3):666.
24. Feng Q, Chen WD, Wang YD. Gut Microbiota: An Integral Moderator in Health and Disease. Front Microbiol. 2018 Feb 21;9.
25. Liu Y, Wang J, Wu C. Microbiota and Tuberculosis: A Potential Role of Probiotics, and Postbiotics. Front Nutr. 2021 May 7;8.
26. Horvath A, Leber B, Schmerboeck B, Tawdrous M, Zettel G, Hartl A, et al. Randomised clinical trial: the effects of a multispecies probiotic vs. placebo on innate immune function, bacterial translocation and gut permeability in patients with cirrhosis. Aliment Pharmacol Ther. 2016 Nov 4;44(9):926–35.
27. Youn HN, Lee DH, Lee YN, Park JK, Yuk SS, Yang SY, et al. Intranasal administration of live Lactobacillus species facilitates protection against influenza virus infection in mice. Antiviral Res. 2012 Jan;93(1):138–43.
28. Robak OH, Heimesaat MM, Kruglov AA, Prepens S, Ninnemann J, Gutbier B, et al. Antibiotic treatment–induced secondary IgA deficiency enhances susceptibility to Pseudomonas aeruginosa pneumonia. Journal of Clinical Investigation. 2018 Aug 1;128(8):3535–45.
29. Shehata E, Parker A, Suzuki T, Swann JonathanR, Suez J, Kroon PaulA, et al. Microbiomes in physiology: insights into 21st-century global medical challenges. Exp Physiol. 2022 Apr 14;107(4):257–64.
30. Zommiti M, Feuilloley MGJ, Connil N. Update of Probiotics in Human World: A Nonstop Source of Benefactions till the End of Time. Microorganisms. 2020 Nov 30;8(12):1907.
31. Day RL, Harper AJ, Woods RM, Davies OG, Heaney LM. Probiotics: current landscape and future horizons. Future Sci OA. 2019 Apr 1;5(4).
32. Hajela N, Nair GB, Ganguly NK. Are probiotics a feasible intervention for prevention of diarrhoea in the developing world? Gut Pathog. 2010 Dec 29;2(1):10.
33. World Health Organization. Global Tuberculosis Report 2022 [Internet]. 2022. Available from: http://apps.who.int/bookorders.