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Microbiological Modification Therapy For Acute Medical Presentations Of Generalised Anxiety Disorder

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ABSTRACT

Generalised anxiety disorder (GAD) displays a variety of psychological and physical symptoms. These are difficult to rapidly control with currently available pharmacological options. This review summarises the evidence for an acute use of microbiological modification drugs targeting vagal nerve stimulation.

A systematic search was conducted in Medline, Pubmed, and PschInfo for all pre-clinical and human studies concerning the use of Lactobacillus and Bifidobacterium in the treatment of GAD.

Twenty-five studies were eligible for inclusion. In mice, modification therapy resulted in improved behaviour that was at least non-inferior to standard therapy (antidepressants). Brain histopathology revealed further anti-inflammatory and neuroprotective benefits. Vagotomy abolished the anti-GAD properties of the treatment.

In humans, several species demonstrated significant reductions in palpitations, shortness of breath, headaches, flu-like symptoms, and abdominal pains vs. placebo within 4 to 12 weeks. *B. bifidum, B. lactis, L. acidophilus and S. thermophiles* showed alleviation of physical symptoms to population baseline when added to an antidepressant. No significant side effects were reported in the studies.

As physical symptoms are typically the presenting medical complaint of GAD, there is potential to control the disease with the administration of bacteria-containing pharmaceutical agents as an adjunct to current antidepressant options.

Keywords: generalized anxiety disorder, Bifidobacterium, Lactobacillus, acute medicine, probiotics, microbiome modification

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INTRODUCTION

Presenting complaints associated with generalised anxiety disorder (GAD) are a very common occurrence on an acute medical intake[1]. After excluding life-threatening causes of chest pain, acute abdomen or palpitations, doctors often find themselves in a peculiar and uncomfortable position of assigning these symptoms to anxiety. Fewer than a half will provide any advice or follow-up[2], leaving unresolved problems and generating subsequent emergency department attendances[3].

One of the reasons for discharging GAD patients without any treatment or advice may be a poor understanding of the brain-centric aetiology of the disease[4] and a general feeling of suboptimal treatment results[5]. Indeed, GAD is often thought to be a predominantly psychiatric condition, ignoring the fact that it is a more complex syndrome of endocrine, immune, autonomic and enteric nervous system (ENS) connections [6].

Recent evidence provides further biological explanation for some of the already known facts about the disease. Life trauma commonly associated with GAD can alter the gut microbiome[7] and affect mammalian neurotransmitters[8]. Epigenetic studies show distinct methylation[9] and micro RNA patterns[10] that may be caused by bacteria[11] and subsequently explain the familial aggregation of the GAD traits. Imaging findings suggest that the changes in limbic and somatosensory brain networks resemble a temporary state rather than a permanent feature of GAD[12], which may be because of the gut input to anxiety regulation via the vagus nerve [13, 14].

Thus, the aetiological paradigm has been shifting towards microbial depletion as a predominant source of GAD symptoms. Unfortunately, these patients are also more prone to receiving unwarranted antibiotic therapy[15], which has been consistently linked to increasing incidence of anxiety [16, 17].

Looking for a new solution

In the light of recent developments in the field, it would be logical to investigate microbiological modification therapies as a remedy for acute anxiety presentations. In this review, we will aim to summarise the currently available evidence of microbiological replacement therapies for GAD in both the pre-clinical and clinical settings, with a focus on two most commonly studied groups of agents: Lactobacili and Bifidobacteria. These genes have been previously described as most promising "psychobiotic" agents that have the potential of altering human neurological system via microbiome and have the greatest body of pre-clinical and human research behind them [18, 19].

MATERIALS & METHODS

A search was conducted in MEDLINE and PsychINFO for studies and abstracts published between database inception and May 22, 2020 (Fig. 1). We used a broad set of key words to identify clinical and pre-clinical studies investigating the effects of *Lactobacillus* and *Bifidobacterium* on GAD. Our search terms were: (ANXIETY/ OR anxiety OR ('Generalized Anxiety Disorder' OR GAD) AND (LACTOBACILLUS/ OR BIFIDOBACTERIUM/ OR (Bifidobacterium OR Lactobacillus). Duplicate references were removed electronically as well as manually. Titles, abstracts, and full texts of articles were screened by two reviewers (MAB and LC). We included English-language studies that reported the effect of *Lactobacillus* and *Bifidobacterium* on GAD. Randomised controlled trials, cohort studies, case-control studies, cross-sectional studies, case series, case reports and qualitative studies were included; meta-analysis, reviews and secondary research articles were excluded. We excluded studies based on the following criteria: primary outcome measurement did not include GAD, did not use specific probiotics from search criteria, and where the acronym 'GAD' did not mean generalised anxiety disorder.

RESULTS

Pre-clinical studies

Rodent models of generalised anxiety offer an insight into the pathophysiology of the disease and different ways neural circuits can be affected by the microbiome. Naturally, these will carry an inherent flaw of inter-species generalisability. To bridge that gap, various anxiety models were used, be it via chemical induction with antibiotics or behavioural restraint of chronic stress exposure. Once conditioned, rodents are observed in a variety of situations with the aim of eliciting social and behavioural deficits. For example, in a swim test (behavioural despair test), animals are subjected to a threat of drowning. The time until they give up trying to swim can be used as an indirect measure of low mood. Biochemical and histopathological

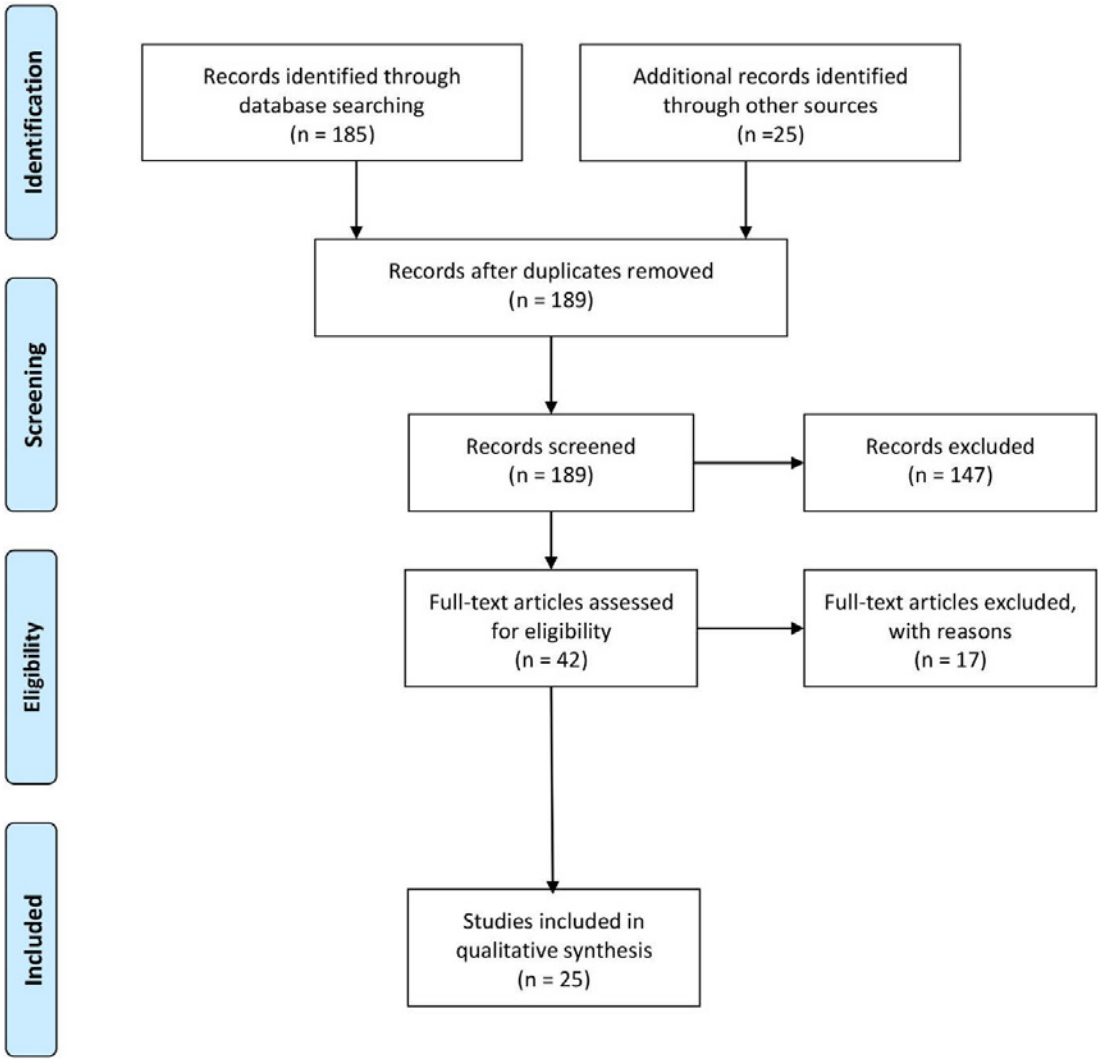

Figure 1. Flowchart of systematic searches and selection of papers eligible for inclusion in the review.

benchmarks of stress or inflammation have also been utilised, in the form of, e.g. hormone levels, inflammatory molecules or microscopic appearances. A summary of all studies is presented in Table 1.

Placebo-controlled

The largest (N=324) study involved a four-week administration of *L. paracasei, L. plantarum* in rodents (starting 1 week prior to stressful stimulus)[20]. Whilst displaying significant reductions in behaviour, the biochemistry results were less encouraging, with some level of gamma aminobutyric acid (GABA) receptor changes but no brain-derived neurotrophic factor (BDNF) neuroprotection.

L. musosae and B. longum seem to be offering additional systemic benefits. In the N=48 experiment, a range of stress-related molecules appeared to be attenuated, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) [21]. Similar results were achieved with *L. reuteri and B. adolescentis* [22].

Interestingly, a preconditioning effect was also observed[23]. In N=22 mice subjected to early life stress *L. plantarum* was superior to placebo after 4 weeks especially in individuals who were noted to be microbiologically dysregulated to start with. Replacing lacking bacteria in these individuals resulted not only in reduced depression-like behaviour but also decreased levels of corticosterone (rodent "stress hormone") and improved functionality of the brain in prefrontal cortex, a region crucial for higher decision-making and emotional processing in connectomics (functional and structural brain connections) of GAD in humans (Fig. 2).

The antibiotic or hyperammonia-induced anxiety

Table 1. Summary of experiments done in rodents, separated by commas, grouped by species.

Species	Dose	Length	Behavioural benefits	Neuroprotection	Modulated immune response	Connectomics	Refs
Lactobacillus							
L. paracasei	1×10^9 CFU	4, 6 weeks	++ vs placebo, vs SSRI	- + via BDNF	- -	- +hippocampal neurochemistry	[20, 27]
L. plantarum	1-2 $\times 10^9$ CFU 1×10^8 CFU	4 weeks 2 weeks	+++ vs placebo, non-inferior to SSRI	--	- + via cytokines + via corticosterone + antioxidant activity	-- + hippocampal neurochemistry + prefrontal cortex neurochemistry	[20, 23, 28, 29]
L.mucosae	1×10^9 CFU	1 week	++ vs placebo, similar to SSRI	+ via BDNF	+ via corticosterone and cytokines	-	[21]
L.reuteri	1×10^9 CFU	1 week	+ vs placebo	++ via BDNF	++ via cytokines and corticosterone	- + hippocampal neurochemistry	[22, 24]
L.helveticus	1-3 $\times 10^9$ CFU	2 weeks 4 weeks	+++ vs placebo, superior to SSRI in some tests, similar to diazepam	--	-+++ via cytokines, cortisol, similar to SSRI	-+ hippocampal neurochemistry	[26, 29, 32, 45]
L.rhamnosus	1×10^9 CFU	4 weeks	+ similar to SSRI	-	-	-	[17]
L.fermentum	1×10^9 CFU	6 weeks	+	-	-	-	[25]
Bifidobacterium							
B.adolescentis	0.25-1 $\times 10^9$ CFU 1×10^8 CFU	3 weeks 2 weeks	+++ vs placebo, superior to amitriptyline, non-inferior to SSRI	-++ via BDNF	+++ via cytokines and corticosterone, antioxidative effect	---	[22, 28, 31]
B.longum	1-2 $\times 10^9$ CFU	3 days 1 week 2 weeks 4 weeks	+++ vs placebo, similaror superior to SSRI in some panels, abolished after vagotomy, similar to diazepam	++ via BDNF ---	---+++ via corticosterone and cytokines	----- + hippocampal neurochemistry + limbic system	[14, 21, 29, 32-34, 46]
B. breve	1×10^9 CFU	4 weeks	+ superior to SSRI in some panels	-	-	-	[46]

Minus = not superior, Plus = better than comparative agent, SSRI = Selective Serotonin Reuptake Inhibitor, BDNF = Brain-Derived Neurotrophic Factor, CFU = Colony Forming Unit.

also responded well to microbiological replacement. *L. reuteri* reduced anxiety, increased BDNF neuroprotection, and suppressed stress-related damaging NF-kb activation in hippocampus. It also alleviated antibiotic-induced colitis in these subjects [24]. Similar results were obtained by N=30 *L. fermentum* [25] and N=18 *L. helveticus* [26] experiments.

Replacement vs. standard treatment

After encouraging, albeit biochemically heterogeneous results of the placebo studies, the attention shifted onto comparing biological replacements with current standards of GAD treatment, both acutely (benzodiazepines) and in long-term (selective serotonin reuptake inhibitors, SSRIs, antidepressants) (Fig. 3).

L. paracasei demonstrated a behavioural response that was superior to fluoxetine [27] and similar in attenuating costicosterone-induced brain damage in

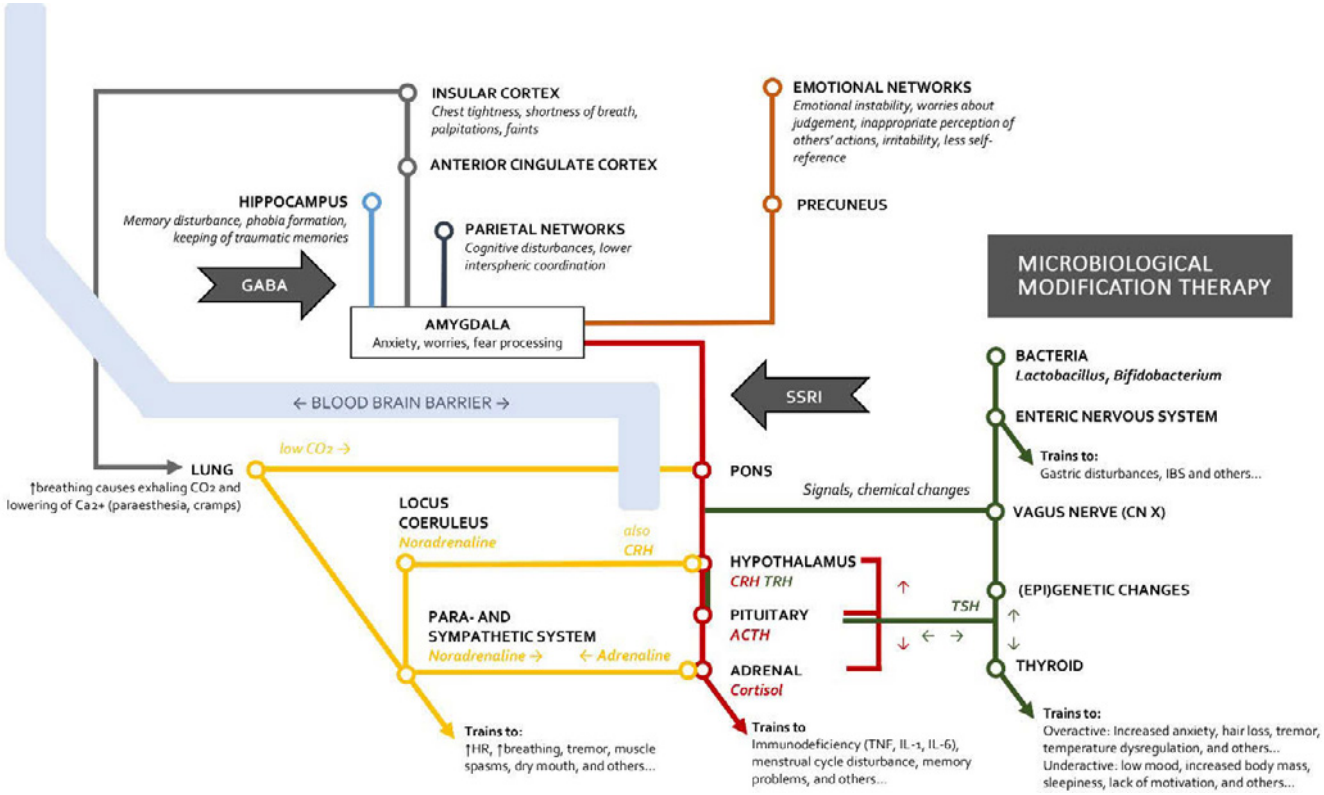

Figure 2. Effects of Bacteria and the Enteric Nervous System on the stylised map of selected neurological interactions that produce physical symptoms of the generalised anxiety disorder. Map not to anatomical scale. Grey arrows indicate where previously studied pharmaceuticals affect the brain connectomics.

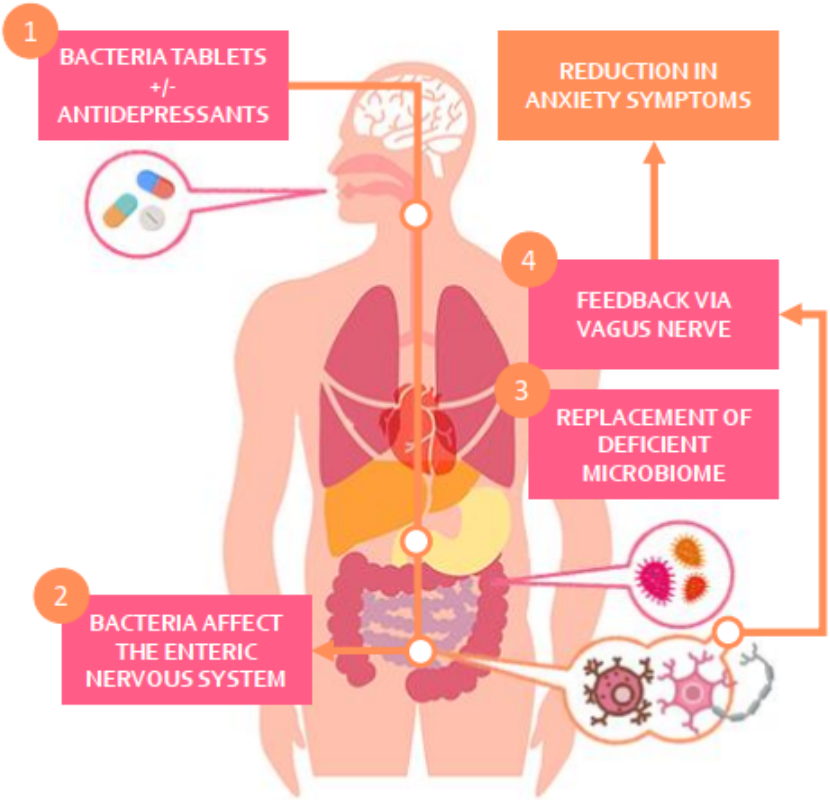

Figure 3. Process of pharmaceutical replacement and modification of the human microbiome with the aim of reducing anxiety symptoms via vagus nerve feedback.

the hippocampal regions. *L. plantarum* was also associated with results non-inferior to that of fluoxetine [28].

A more mixed response came out of a combined study of *L. helveticus, L. plantarum,* and *B. longum.* Some but not all behavioural instruments reported bacterial superiority, with one assigning more efficacy to the SSRI. Central nervous inflammation and damage was lower in the microbacterial arm, however [29]. This was also the case in the *L. rhamnosus* study [17] or *B.longum and B.breve* vs. escitalopram experiment [30].

When compared to the tricyclic antidepressant, amitriptyline *B. adolescentis* generated better behavioural, neuroprotective and inflammatory responses than standard treatment [31]. *B.longum* and *L.helveticus* have similar effects on mice when compared to diazepam [32].

Vagus nerve implications

The microbiological pathogenesis of GAD relies heavily on the vagus nerve as the main communicating pathway between the enteric and central nervous systems. To investigate this connection, conventional experiments of biological replacement were enhanced by the inclusion of the vagotomy group.

In the colitis stress-induced model, *B.longum* exhibited anxiolytic effects that were abolished by performing vagotomy as opposed to sham neurosurgery [33]. *L.rhamnosus* additionally demonstrated an improved neurochemistry in the hippocampus, cingulate and pre-limbic cortices, which are areas involved in central emotional and sensory processing. These effects were again abolished by disruption of the vagus nerve [14]. This vagal effect was, however, not observed in the colitis model of *L. rhamnosus* and *B.longum* [34].

Human studies

The initial optimism of pre-clinical experiments was significantly scrutinised by the last decade of human studies. A recent meta-analysis of 1527 participants across 14 papers show very little difference in symptoms or quality of life after probiotic treatment in healthy or irritable bowel syndrome (IBS) subjects [35]. This somewhat confirms the pre-clinical observations that well-balanced microbiomes of healthy patients do not require, and thus will not benefit from, a biological replacement. An inquiry was thus launched in the more specified segment of anxiety disorders (Table 2).

Placebo-controlled

L.plantarum demonstrated placebo-level reductions in stress-measuring inventories depression anxiety severity score 42 (DASS-42) score in perceived stress scale 10 (PSS-10)-confirmed moderately stressed humans [36]. Interestingly, the real superiority of the drug was observed in the symptomatic domains, where biological replacement achieved normalisation of palpitations, breathlessness, or fear of the unknown. Some evidence of TNF-α inflammatory alleviation was also noted.

A similar preference to physical symptoms was observed in a study of stressed Japanese medical students where *L. casei shorta* lowered cortisol and resolved abdominal pain and flu-like symptoms in the 8 weeks leading up to the final examinations vs. placebo [37]. *B. longum* seem to be exhibiting the same effect on physical symptoms of patients with comorbid IBS [38]. These species also contributed to IBS symptom relief and reduced amygdala activity on functional MRI. No change in the State-Trait Anxiety Inventory (STAI) was noted in the studies, however.

On the other hand, lower size effects were observed after administration of *L.gasseri* in another group of stressed medics [39]. Whilst statistical changes on STAI, general health questionnaire 28 (GHQ-28) questionnaires were observed, these would bear little clinical significance. Even after reductions, the subjects would be considered stressed on the instruments. Further investigation of gut make-up revealed that probiotics preserved *Bifidobacteirum* colony whilst limiting the proliferation of *Streptococcus,* which may indicate further microbiological intricacies in generating clinical benefit.

Similar observations were made in another study where despite scoring as anxious on self-reported questionnaires, not all participants may have a biochemically or microbiologically proven GAD. Again, in cases of an already balanced state, replacement offers very little benefit [40].

Comparison to standard treatment

It is envisaged that in clinical practice, most GAD patients will receive their standard treatment first. Studies comparing biological replacement to SSRIs are thus of particular clinical interest.

Table 2. Summary of experiments done in humans grouped by species.

Species	Dose	Duration (First effects)	Behavioural benefits	Cognitive benefits	Physiological changes	Inflammatory benefits	Neuroprotection	References
VS. PLACEBO								
L. plantarum	2×10^{10} CFU/day	12 weeks (4 weeks)	DASS-42 anxiety from moderate to normal	No effect	Normalisation of palpitations, dyspnoea and fear	+ via cortisol, cytokines	Not studied	[36]
L. gasseri	1×10^{10} CFU/day	24 weeks (12 weeks)	Mild or no reductions on questionnaires	Not studied	Attenuation of bacterial flora depletion	+ via chromogranin A but no change in cortisol	Not studied	[39]
B. longum	1×10^{10} CFU/day	6 weeks	Mild improvement in depression HAD-D. No difference in anxiety	No effect	Normalisation of physical functioning (activities of daily living)	No effect	Improved amygdala activity	[38]
L.casei shorta	1×10^{10} CFU/day	8 weeks	No change in STAI	Not studied	Normalisation of flu-like symptoms, headaches, functional abdominal pain	+ via cortisol	Not studied	[37]
L. acidophilus, B.animalis, L. paracasei, L. casei, Bacillus coagulans	2×10^{10} CFU/day	6 weeks	Not studied	Not studied	Mild effect on abdominal pain, no effect on other symptoms	Mild isolated effect on IgA, otherwise ns	Not studied	[40]
Vs. Standard treatment								
B. bifidum + *B. lactis* + *L. acidophilus*	18×10^{9} CFU/day sertraline 25mg	8 weeks (4 weeks)	Mild superiority on state but not trait anxiety, and not on BAI	Not studied	Reduction from severe to mild anxiety systemic symptoms on HAM-A [SSRI+pacebo alone reduced to moderate]	No effect	Not studied	[41]
L. acidophilus, B. bifidum, S. thermophiles	600 mg magnesium orotate + 10-6 bn CFU as an addition to SSRI Vs. no control group	16 weeks (8 weeks)	Reduction from severe to mild depression on BDI	Overall BDI reduction	Overall BDI reduction	Not studied	Not studied	[42]

Plus = better than comparative agent, SSRI = Selective Serotonin Reuptake Inhibitor, DASS-42 = Depression Anxiety Stress Score-42, HAD=Hospital Anxiety Depression Score, BDI = Beck Depression Inventory, CFU = Colony Forming Unit.

The largest trial to date compared *B.bifidum, B.lactis* and *L.acidophilus* (Fig. 4) with standard dose sertraline (25mg) to sertraline with a probiotic placebo in patients with a confirmed GAD diagnosis [41]. As in the previous studies, the biological replacement affected mainly somatic (cardiovascular, gastrological, neurological, etc.) symptoms. Participants treated with SSRI alone reduced the severity of disease by a third, whilst patients receiving combination therapy halved their symptoms, taking them to a "mild" tier level on HAM-A score. Similar changes were observed in state STAI; Beck anxiety inventory (BAI) and trait STAI anxiety were lowered in both groups similarly. The effect

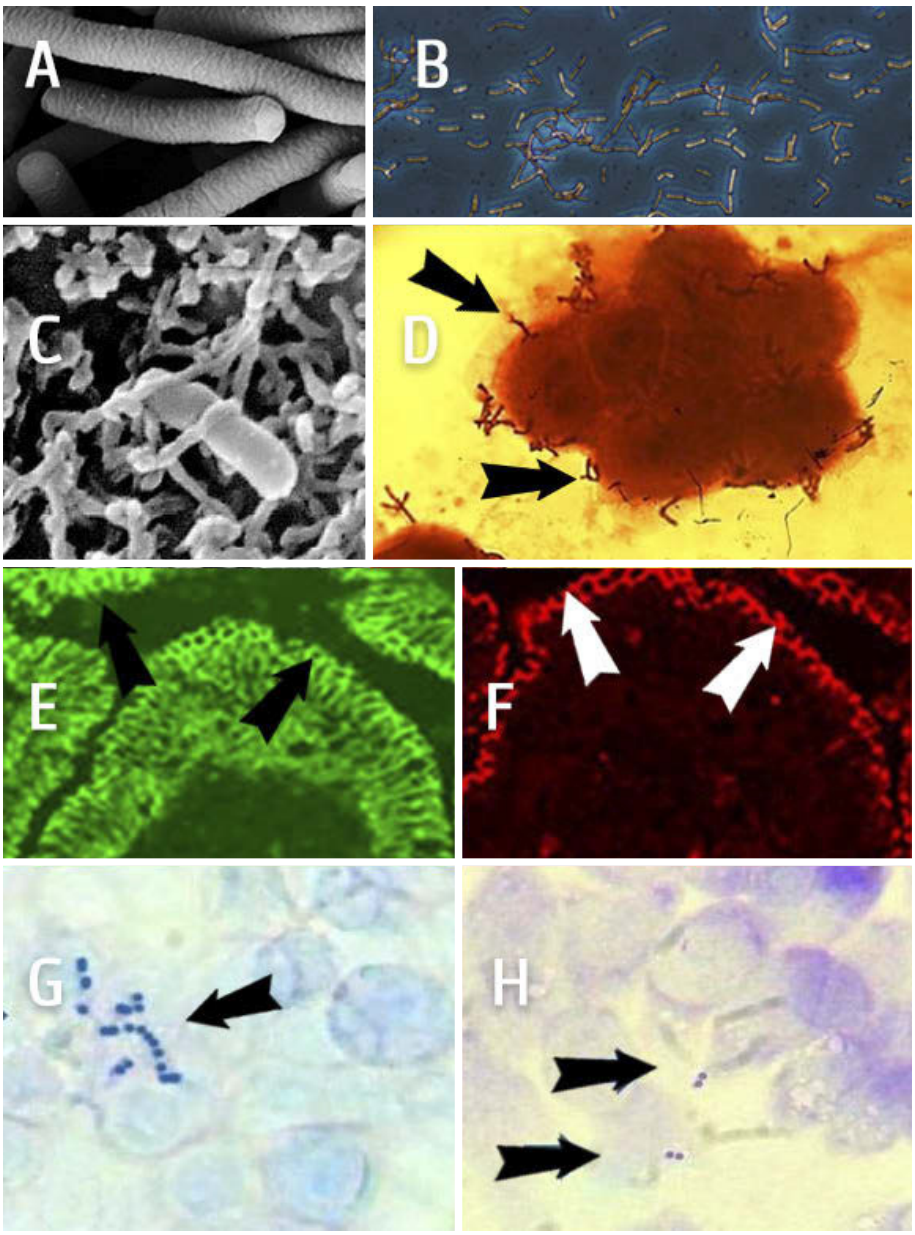

Figure 4. Micrographs of agents successfully used in humans (arrows pointing at bacteria). (A) Electron micrograph of L. acidophilus (Mogana Das Murtey and Patchamuthu Ramasamy) (B) L.acidophilus 6000× negative phase contrast micrograph (Josef Reischig) (C) B. bifidum adhered to cell 1000× micrograph (Seockmo Ku et al.) (D) B. bifidum interacting with microvilli 20000× crystal violet stain (Seockmo Ku et al.) (E) B. lactis stained by ZO-1 and (F) E-cadherin in jejunal epithelium (Marie C. Lewis et al.) (G)(H) Two strains of S. thermophilus (Armin Tarrah et al.) Published under CC-BY.

was also weaker in patients already making positive dietary changes.

Another study showed significant improvements in Beck depression inventory (BDI) and quality of life scores in patients with SSRI-resistant depression and comorbid anxiety treated with *L.acidophilus, B. bifidum, and S. thermophiles* (Fig. 4) with magnesium [42]. This study, however, suffered from lack of a separate magnesium arm and it was not blinded.

DISCUSSION

Microbiological modification therapies are novel and emerging treatment modalities for GAD. As

such, they face several important challenges. First, inducing a complex neurological condition in the constraints of a few weeks' rodent experimentation is incredibly challenging. Surrogate settings and anxiety inducers can be utilised, but results vary depending on the behavioural or biochemical parameters chosen.

Furthermore, whilst there seems to be consensus as to the Lactobacillus and Bifidobacterium genera, the actual species are largely varied across different studies. It is not inconceivable to suppose that they may exhibit various effects, be it synergetic or exclusionary on the gut-brain-axis.

Rodent models supply important pieces of the pathophysiological puzzle of GAD. They allow for invasive histopathological examination of the nervous system, which provides further evidence for already hypothesised pathways involved in generating anxiety states. The studies on vagotomy are especially interesting as they show a direct causal relationship between changes in gut flora and the central nervous response.

The challenges of pre-clinical studies are even more pronounced in human experiments. The initial surge of healthy subject papers very quickly verified the "one-fits-all" approach. Whilst there may be minor improvements in mood and anxiety for microbiomically balanced individuals, the true power of probiotics seems to lie in replacement of distorted gut flora.

Studies should thus scrutinise participants either by stringent GAD diagnostic criteria, biochemical measurements, or, ideally, careful examination of the individual gut flora composition and metabolic profiling. With the advent of modern mass analysing options, this may soon become more commercially viable.

Another potential route of innovation would be through studying different paths in the gut-brain axis. There is a myriad of epigenetic, biochemical, inflammatory, and enteric agents that could utilise the same signalling system. A new drug class can be envisaged that imitates the role of biological replacements by providing molecules produced by the bacteria or by stimulating receptors at the vagus nerve termini. This, however, remains to be further elucidated by research.

CONCLUSIONS

There remains an important clinical question: is there enough evidence to recommend the use of biological replacement therapy in GAD patients? From available evidence, unlike SSRIs, no adverse effect was reported in any of the 1349 patients receiving probiotics in the studies [43]. Furthermore, bacteria seem to predominantly target the somatic symptoms, which tend to be the primary acute medical concerns of the patients with GAD. Small studies included in this review show significant effects in normalising these symptoms to the population level and demonstrate an additional benefit, on top of the conventionally used SSRIs or benzodiazepines.

These products are widely available in the consumer market and are considered safe to use by public bodies and regulators [44]. There is an adequate and growing body of evidence suggesting their pathophysiological significance and with the advent of connectomic understanding of GAD, gut-associated vagal stimulus seems to be making more sense both clinically and scientifically.

Thus, patients who are already established on standard treatment and may wish to achieve additional somatic benefits in the short to medium term may benefit from a prescription of biological replacement therapy. Although it is likely that before full metabolic profiling is commercially available, the benefits and confidence with which they are recommended will remain limited.

CONFLICT OF INTEREST

None declared

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