Reviewer's report

Title: Probiotics and oxytocin nasal spray as neuro-social behavioral interventions for patients with autism spectrum disorders: a pilot randomized controlled trial protocol

Version: 0 Date: 24 Jun 2019

Reviewer: Amanda Whippey

Reviewer's report:

Reviewer Comments Re: Probiotics and oxytocin nasal spray as neuro-social behavioural interventions for patients with autism spectrum disorders: a pilot randomized controlled trial protocol

Major Comments

1. ASD symptom severity varies widely between patients. The DSM V uses a 3 level severity score to describe this spectrum. There is no mention made of documenting ASD severity. This is a very important potentially confounding factor. As the severity of autism can be categorized may influence the effect of the probiotic, I would encourage the authors to ascertain this information at the time of enrollment and consider stratified randomization.

2. This goal of this article is to define a protocol for a pilot trial that will look at the feasibility for a larger RCT however feasibility criteria are poorly defined throughout the article. Specific recruitment rates, adherence rates, data collection goals which would indicate feasibility need to be set.

3. Inclusion criteria indicates that a pre-existing diagnosis of autism and a confirmation of diagnosis by DSM V criteria, ADOS and/or ADI-R is required. Why are multiple diagnostic criteria being used? As written, it appears that both the DSM V and ADOS are required - did you mean one of the criteria were required? Who will confirm the patient meets criteria?

4. Inclusion criteria describes willingness to provide blood samples, will this introduce a selection bias towards high functioning patients?
5. A 12 week treatment course of probiotics was selected, please provide rationale and reference for the selected duration.

6. Reporting guidelines for nasal oxytocin administration (Guastella et al 2013) include all ingredients, preservatives, pH, bottle design and training. Patients with sensory issues may need training/practice prior to the start of the study. Please add to methods.

Minor Comments:

[P1,L7] - Remove (ASD) from title

[P2,L21] - "This paper presents a protocol for a pilot randomized controlled trial testing oral supplementation of L. reuteri probiotics and intranasal OXT spray." - should indicate what outcome you are assessing.

[P2,L26] - Recruitment rate is not mentioned as a feasibility criteria

[P2,L41] - name validated behavioural questionnaires used

[P2,L46] - please clarify "GI function" as secondary outcome

[P4,L14] - "some pathophysiological findings may shed light on its effective treatment" - without looking up references, this statement is quite vague. Can you do more to inform the reader?

[P4,L36] -"Andari et al reported a small randomized placebo-controlled trial wherein, …"

[P6,L24] - "There is a need for at least 30 participants.." - awkward, please revise

[P6,L45] - IRB - spell out acronym the first time

[P7,L27] - "Able to attend 3 clinic visits"

[P8,L33] - Indicate MGH acronym prior to use

[P8,L38] - please clarify "international participants" - vague

[P9,L20] - be consistent in your use of probiotics or L. reuteri

[P9,L42] - "Syntocinon intranasal spray (Novartis pharmaceuticals) will be used"

[P10,L17] - Include that both behaviour checklists are validated in ASD

[P11,L21] - "relative abundance of different bacterial taxa" - Scale is indicated as 0-100%, what is the normal value or reference range?
Baseline information - vague. What collection tool will you use for past medical history, what questions/questionnaires will assess immune function and lifestyle factors? Please specify.

as mentioned above, please specify targets for feasibility criteria.

"pilot and feasibility study that explores the implementation … and in combination with exogenous OXT administration as a treatment for ASD symptoms."

"designed to investigate the synergistic.."

"whole blood 5-HT levels in ASD subjects.."

"probiotic induced endogenous OXT in ASD"

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