Treating gastrointestinal disorders in children with autism using Microbiota Transplant Therapy (MTT)

James B. Adams
Arizona State University
http://autism.asu.edu

Co-Authors: Dae-Wook Kang, Ann C. Gregory, Thomas Borody, Lauren Chittick, Alessio Fasano, Alexander Khoruts, Elizabeth Geis, Juan Maldonado, Sharon McDonough-Means, Elena L. Pollard, Simon Roux, Michael J. Sadowsky, Karen Schwarzberg Lipson, Matthew B. Sullivan, J. Gregory Caporaso; Rosa Krajmalnik-Brown
Disclosure Slide

Several authors (JBA, D-WK, RKB, TB, AK, JGC, and MJS) have pending/approved patents related to the use of FMT and/or probiotics for various conditions including autism. MJS, AK, JBA, RKB, and D-WK have received research funding from Crestovo for FMT research. JBA, JGC, RKB, and MJS are part-time consultants for Crestovo. The other authors do not have any financial conflicts of interest.

JBA is also president of a non-profit, Autism Nutrition Research Center, which produces ANRC Essentials, a vitamin/mineral supplement for children and adults with ASD.
GI problems

• Gastrointestinal problems are common in children and adults with autism

• Most studies show about 30-50% have chronic constipation, diarrhea, or alternating diarrhea/constipation
Gut Problems associated with worse symptoms (all four areas) – Adams et al 2011

Autism - Low 6-GSI

Autism - High 6-GSI
Investigated 499 children ages 2-5 years with ASD vs. 324 controls;

• Children with ASD 8x as likely to have 1 or more frequent GI problems compared to controls (15% constipation, 13% diarrhea for ASD vs. 4%/2% for controls)

• Found ASD children with GI problems had increased irritability, social withdrawal, stereotypy, and hyperactivity
Autism and gut microbiome

**INTESTINAL MICROFLORA**

- 10^{14} micro-organisms, >500 different species
- Stomach: \(10^2\) to \(10^3\)
- Duodenum: <\(10^{4-5}\)
- Jejunum
- Ileum: \(10^3\) to \(10^7\)
- Colon with appendix: \(10^9\) to \(10^{12}\)
- Enterobacteria
- Enterococcus
- Faecalis
- Bacteroides
- Bifidobacteria
- Peptococcus
- Peptostreptococcus
- Ruminococcus
- Clostridia
- Lactobacilli

![Image of intestines and microorganisms]

**Time Magazine Cover**

**Inside the Hidden World of Autism**

By Claudia Wallis
We Are not Alone

The Human Microbiome Project says the human body has 100 trillion microscopic life forms living in it.

You call this living?
What is the role of human gut microbiota?

- Break down plant polysaccharides
- Promoting GI motility
- Producing vitamins
- Competing against pathogen
- Convert fiber to short-chain fatty acids (which feed colon cells)

- GI problems
- Producing toxins
- Disrupting immune system
- Competing against commensal bacteria
Abnormal Oral Antibiotic Use

• Five studies reported 2-3x higher usage of oral antibiotics during infancy of children with autism vs. controls, usually for treating ear infections.
• Commonly used oral antibiotics eliminate almost all of the normal gut bacteria.

Biotin

• A study of vitamin levels in 55 children with autism vs. 44 controls found that the primary difference was children with ASD had 20% lower levels of biotin, p<0.001. (Adams et al 2011)

• Also, the degree of improvement due to a vitamin/mineral supplement was primarily associated with low levels of biotin and vitamin K. (Adams et al 2012)

• Both biotin and vitamin K are primarily made by beneficial gut bacteria.
Antibiotic Therapy

A small open-label treatment study by Sandler et al with a potent non-absorbable antibiotic (Vancomycin) found temporary improvement in gut function and behavior, but the gains were lost when the treatment was stopped.


ARI Survey of Parent Ratings of Treatment Efficacy:

<table>
<thead>
<tr>
<th></th>
<th>% Worse</th>
<th>% No Change</th>
<th>% Better</th>
<th>Number of Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antifungals\textsuperscript{C}: Diflucan</td>
<td>5%</td>
<td>41%</td>
<td>55%</td>
<td>330</td>
</tr>
<tr>
<td>Antifungals\textsuperscript{C}: Nystatin</td>
<td>5%</td>
<td>46%</td>
<td>49%</td>
<td>986</td>
</tr>
<tr>
<td>Antibiotics (not recommended)</td>
<td>31%</td>
<td>57%</td>
<td>12%</td>
<td>1799</td>
</tr>
</tbody>
</table>
Yeast in autism?

Many anecdotal reports of yeast overgrowth in children with autism, and limited research evidence. However, Adams et al 2011 did not find elevated yeast (by culture or microscopically) in study of 58 children with autism vs 39 controls, and yeast overgrowth very rare on endoscopies (<10%) per anecdotal physician reports.
## Cultured & Microscopic Measurements of Yeast in ASD vs. Controls

<table>
<thead>
<tr>
<th></th>
<th>ASD</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultured Yeast</strong></td>
<td>28%</td>
<td>13%</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Microscopic Yeast</strong></td>
<td>71%</td>
<td>85%</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
Two small studies by Finegold et al found some limited evidence of abnormal anaerobic bacteria, primarily increases in clostridia. A study by Parracho et al also found increased amounts of clostridia.


One study of 58 children with autism vs. 39 controls found some abnormalities in gut bacteria, including decreased levels of bifidobacteria (an important beneficial bacteria) in children with autism compared to controls.

DNA-based methods

Two small studies using DNA-based methods to investigate gut bacteria have been conducted, but yielded dissimilar results – much larger studies are needed.

- Finegold et al (2011) found increased levels of Desulfovibrio bacteria in children with autism.

Less bacterial diversity in autism

- Most humans have about 1000 different bacteria in their guts, and higher diversity is believed to be healthier.
- Our study (Kang et al 2013) found that children with ASD have about 25% less types of bacteria; i.e., 200-300 less species.
- Our study also found children with ASD have unusually low levels of *prevotella*, which is associated with healthy fiber-rich diets.
Prevotella & Diet

A study of African children on traditional neolithic diet (rich in carbohydrate, fiber, non-animal protein). Mostly whole grains (millet grain, sorghum), legumes (black-eyed peas), and vegetables.

53% of gut bacteria were Prevotella, vs. undetectable Prevotella in European children on traditional western diet.
Treatment Implications

• Broad-spectrum probiotic probably needed
• Unfortunately, most commercial over-the-counter “probiotics” only contain a few bacteria, and not the ones needed by children with autism, and fail to implant long-term
• Suggests transplant of beneficial bacteria from healthy donor may be useful
Probiotics for ASD?

- Parracho et al 2010 – randomized, double-blind, placebo-controlled cross-over study with *lactobacillus plantarum* (45 Billion CFU/day) in children with ASD (most without GI problems?)
- 62 participants recruited, 23 dropped out at baseline due to difficulty collecting stool samples, 22 dropped out during the study (including 3 for adverse events – skin rash, diarrhea, weight loss), so only 17 completed the study.
- Slight improvement in formed stools for probiotic vs. placebo:

<table>
<thead>
<tr>
<th>Stool Consistency at end of study</th>
<th>Probiotic (n=8)</th>
<th>Placebo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft</td>
<td>19%</td>
<td>19%</td>
</tr>
<tr>
<td>Formed</td>
<td>73%</td>
<td>65%*</td>
</tr>
<tr>
<td>Hard</td>
<td>8%</td>
<td>16%*</td>
</tr>
</tbody>
</table>

- No significant effect on abdominal pain (rare problem), bloating (rare), flatulence (common)
- Slightly better improvement on “problem behavior”, but probably not statistically significant (no comparison of probiotic vs. placebo)
2nd Probiotic Study

West et al 2013 – open-label study of Delpro (mixture of 5 probiotics, 30 billion CFU/day) for 6-month treatment

- *Lactocillus acidophilus, Lactobacillus casei, Lactobacillus delbruecki, Bifidobacteria longum, Bifidobacteria bifidum*; 6 billion CFU/day each
- 33 participants started, 25 completed;
- 84% had moderate/severe constipation, and 56% had moderate/severe diarrhea
- 20% reduction in ATEC score, p<0.05; significant improvement in all 4 subscales – speech, sociability, sensory/cognitive, physical/behavior
- Diarrhea: 48% improved, 32% no change, 8% worse
- Constipation: 52% improved, 36% no change, 4% worse
3rd Probiotic Study

Kaluzna-Czapinska et al 2012 – Open label study with 22 children with ASD

• Lactobacillus acidophilus (strain Rosell-11), 10 Billion CFU/day for 2 months
• Urinary d-arabinitol (metabolite of candida) decreased 50%
• Some reported improvements in ability to concentrate (71%), eye contact (41%), ability to follow orders (77%), ability to react to other peoples emotions (36%)
4th Probiotic Study

Tomova et al 2015: open label study involving 10 children with ASD given ‘Children Dophilus”
3 strains of Lactobacillus (60%), 2 strains of Bifidumbacteria (25%) and one strain of Streptococcus (15%) – 3x/day for 4 months

Effect of probiotic:
• Firmicutes significantly decreased, which resulted in the increase of the Bacteroidetes/ Firmicutes ratio to the level of the healthy individuals
• Bifidobacterium decreased significantly after the probiotic intervention to the level of the healthy subjects.
• Desulfovibrio — a suspected pathogen in autism, decreased significantly

No description of effect on symptoms
Probiotics
- data from national survey of parents

Overall Benefit Score
- Overall Adverse Score

Most Common Adverse Effects

Most Benefited Symptoms

n = 176
Summary of Probiotic Research

1) Parracho et al 2010 – randomized, double-blind, placebo-controlled cross-over study with lactobacillus plantarum; 73% dropped out; possibly minor GI and ASD benefits

2) West et al 2013 – open-label study of mixture of 5 probiotics; some improvement in constipation, diarrhea, and ASD.

3) Kaluzna-Czaplinska et al 2012 – Open label study with Lactobacillus acidophilus; decreased d-arabinitol (metabolite of candida), possible improvement in ASD.

4) Tomova et al 2015: mixture of 6 bacteria; some alterations in gut bacteria, unknown effect on symptoms.
Fecal Transplant

• Approximately half of stool is bacteria.
• The US Food and Drug Administration (FDA) allows FMT for use in patients with a dangerous GI infection called clostridium difficile (CD) when other therapies fail. CD kills about 15,000 people in the US each year, and hospitalizes many more.
• A recent review of 27 published scientific case reports and studies involving 317 patients with life-threatening, antibiotic-resistant CD found that FMT therapy resulted in an overall cure rate of 92%, usually with a single dose, with usually little or no side effects.
• The amazing success of FMT therapy for CD infections has led to its investigation for treating many other GI problems.
Fecal Transplant (cont.)

- FMT in ulcerative colitis (UC) *in sporadic cases* can resolve symptoms within 6 weeks and maintain remission up to 13 yr (9 patients; Borody 1989, 2001, 2003, Bennet 1989). Occasional sporadic ‘cure’ differs from remission.
- And FMT in UC improved stool frequency and abdominal pain in 7/8 patients (88%) - degree of benefit varied (Brandt 2012).
- FMT for Crohn’s disease was reported to alleviate symptoms within 3 days in a few cases (Borody 1989)
- FMT for IBS and IBD involved a case series of 55 patients, with a cure rate in 20 (36%), decreased symptoms in 9 (16%) and no response in 26 (47%) (Borody 1989).
Fecal Transplant (cont.)

FMT for chronic constipation involved a series of 45 patients, and a combination of colonoscopic FMT followed by fecal enemas resulted in improvements in defecation, bloating, and abdominal pain in 40 (89%). At follow-up 9-19 months later, defecation remained normal in 60% (Andrews 1995).

Would it also help for chronic constipation in autism?

Probably, since we suspect chronic constipation is often due to pathogenic bacteria, but research is needed to be sure.
Microbiota Transplant for Autism

One of our collaborators, Dr. Thomas Borody, a gastroenterologist from Australia, has used FMT treatment nearly 5000 patients, including 9 children with autism treated with a culture of 20 gut bacteria, for which he reports:

”After 3 months of the cultured microbiota there was a substantial improvement in bowel function, and the parents noticed a substantial reduction in the odor of their stool. Several children started the study with abdominal cramps which disrupted their sleep, and by the end of the treatment their abdominal cramps were reduced and they were able to sleep throughout the night. Parents reported marked increase in vocabulary, clear improvement on task performance and new ability to listen to parents’ requests.”
Trial of MTT for Autism

The FDA and ASU’s Human Subject Board approved our pilot study of 18 children with autism ages 7-16 years to participate in a trial of MTT.

Study Goal:
Evaluate the safety and tolerability of a combination of oral vancomycin (an antibiotic) followed by a microbiome transplant (transferring gut microbiome from a healthy person) on children with autism who have gastrointestinal problems.
Our Microbiota Transfer Protocol

• 2 weeks oral vancomycin
• 1 day fasting and bowel cleanse
• High dose microbiota (oral or rectal) & prilosec
• 7-8 weeks low dose microbiota (oral) & prilosec
Microbiota Source

• Full-spectrum microbiota provided by Crestovo
• Collected from carefully-screened very healthy donors (90% of general population rejected)
• Purified extensively so that only microbiota remained (FMT generally done with raw stool)
• Essentially a super-probiotic of 1000 species (vs. 1-10 for standard probiotics)
Participants

18 autism participants
Ages 7-16 years
Moderate/severe GI problems
  Constipation (and sometimes diarrhea): n=15
  Diarrhea only: n=3
Excluded very low functioning children who could not assent.
Compliance

• All 18 children completed the study (no drop-outs)
• MoviPrep was difficult to tolerate, and some did not drink all of it; in a few cases fasting was not complete
• Oral high-dose MT well-tolerated in 12 of 13 – 1 switched to rectal
• Rectal high-dose MT well-tolerated in 6 of 6, and maintained for several hours or longer
• Oral low-dose MT very well tolerated – no problems.

Safety

• Vancomycin – 1 case of rash
• Microbiota: No significant adverse side-effects
• No long-term concerns re. ChemPanel or CBC (Complete Blood Count)
Vancomycin: temporary adverse effect

12 of the 18 children had a behavioral reaction to the vancomycin. Started 1-4 days after the start of the vancomycin, and lasted 1-3 days in most cases, although 1 participant had symptoms lasting for 3 weeks.

- 7 cases: mild to moderate increase in hyperactivity
- 5 cases: mild to moderate increase in tantrumming/aggression.

After the symptoms disappeared, GI symptoms and autism symptoms began improving.

Reaction primarily occurred in those with high ABC scores.

Very similar to Sandler 2000 study

We hypothesize this reaction is due to bacterial toxins being released when the vancomycin kills off harmful bacteria.
Gastrointestinal Symptom Rating Scale (GSRS) – scale 1-7

Vancomycin | MT | No treatment

82% reduction in GSRS by week 10, p<0.001
GSRS – individual response

16 children: 70-100% improvement
1 child: 30% improvement
1 child: no change (incomplete bowel cleanse)
GSRS Subscales

On average, GI improvements maintained 8 weeks post-treatment
<table>
<thead>
<tr>
<th>Type 1</th>
<th>Separate hard lumps, like nuts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2</td>
<td>Sausage-like but lumpy</td>
</tr>
<tr>
<td>Type 3</td>
<td>Like a sausage but with cracks in the surface</td>
</tr>
<tr>
<td>Type 4</td>
<td>Like a sausage or snake, smooth and soft</td>
</tr>
<tr>
<td>Type 5</td>
<td>Soft blobs with clear-cut edges</td>
</tr>
<tr>
<td>Type 6</td>
<td>Fluffy pieces with ragged edges, a mushy stool</td>
</tr>
<tr>
<td>Type 7</td>
<td>Watery, no solid pieces</td>
</tr>
</tbody>
</table>
Stool Diary Results -
% days abnormal/no stool

Stool normalized from “too hard” or “too soft” towards “just right”
Autism symptoms steadily improved during treatment, then maintained at 8 weeks post-treatment.
Parent Global Impressions (cont.)

At end of treatment, the Overall Autism/Related symptoms were scored by the parents as:

- Much Better – 4
- Better – 8
- Slightly Better – 5
- Little/No change – 1
### Parent Global Impressions - subscores

<table>
<thead>
<tr>
<th>2.2</th>
<th>Stools/GI Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
<td>Expressive Language/Speech</td>
</tr>
<tr>
<td>1.6</td>
<td>Attention/Focus</td>
</tr>
<tr>
<td>1.6</td>
<td>Sociability</td>
</tr>
<tr>
<td>1.4</td>
<td>Receptive Language/Comprehension</td>
</tr>
<tr>
<td>1.4</td>
<td>Cognition/Thinking</td>
</tr>
<tr>
<td>1.2</td>
<td>Play Skills</td>
</tr>
<tr>
<td>1.2</td>
<td>Irritability/Mood</td>
</tr>
<tr>
<td>1.2</td>
<td>Anxiety</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.1</th>
<th>Eye Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Tantrums/Meltdowns</td>
</tr>
<tr>
<td>1.1</td>
<td>Stimming/Perseveration</td>
</tr>
<tr>
<td>1.0</td>
<td>Hyperactivity</td>
</tr>
<tr>
<td>0.9</td>
<td>Aggression</td>
</tr>
<tr>
<td>0.8</td>
<td>Sensory Sensitivity</td>
</tr>
<tr>
<td>0.7</td>
<td>Sleep</td>
</tr>
<tr>
<td>0.7</td>
<td>Self-Abusive</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>1.9</th>
<th>OVERALL Autism/Related Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>average of all scores</td>
</tr>
</tbody>
</table>
23% improvement in CARS, p<0.001
Aberrant Behavior Checklist (ABC)

27% reduction in Total ABC, p=0.001
Significant improvement irritability, lethargy, hyperactivity, and speech (p<0.05)
Microbiome diversity increased after MT (and became more like donor)

Faith PD (phylogenetic community richness)
Summary

Abnormal gut bacteria in children with autism, including lack of beneficial bacteria.

Microbiota Transplant Therapy (MTT) seems like a promising method to restore normal gut bacteria/microbiome, and thereby improve gut symptoms and possibly autism symptoms.

More research, including larger, placebo-controlled studies, needed to verify results (now being planned).

THANKS!

Many thanks to families who participated.

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Thanks to University of Minnesota & Crestovo for microbiota

Thanks to Walgreens for compounding oral vancomycin.