REVIEW ARTICLE

THE LINK BETWEEN AUTISM SPECTRUM DISORDER AND GASTROINTESTINAL MICROBIOTA

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Background: In recent times multiple attempts have been made to search for the link between Autism Spectrum Disorder (ASD) and gut microbiota. This link is not a myth as the microbiota composition and in turn its bi-products affect not only the Gut-Brain axis but also the hypothalamic-pituitary-adrenal (HPA) axis and the immune response. Aim of the study was to find the relation between the Gut Microbiota and the pathophysiology and in turn manifestations of ASD. **Methods:** Eight original articles were identified by a systematic search of the MEDLINE database. Those articles were included in the review with clear mention of ASD and microbiota in titles and abstracts. **Results:** In the majority of studies, Bacteroidetes/ Firmicutes ratio was deranged only one reported it to be normal. Bacteria such as Actinomyces and Proteobacteria were increased and Bacteroides, Bifidobacterium, Lactobacillus, and Prevotella were decreased. The commonest method of sequencing observed was 16S rRNA. **Conclusion:** The microbiota composition of the gut does affect the manifestations of autism spectrum disorder. The derangement of the gut commensals may lead to mood disorders, depression, and other symptoms in autistic kids.

Keywords: Autism spectrum disorder; Gastrointestinal microbiome; Microbiota; Gut

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INTRODUCTION

Autism spectrum disorder (ASD) is neurodevelopmental delay disorder, that ensues in childhood and constitutes disordered social interaction, and the presence of restricted and repetitive patterns of behaviour that persist throughout life.[1,2] Autistic kids may suffer from a number of ailments, GI symptoms are one of them. Among a spectrum of gastrointestinal (GI) symptoms, constipation and loss of appetite have been the top reported ones among autistic kids. The treatments of ailments associated with ASD usually continue throughout life.[3]

The gut-brain axis is a complex bidirectional axis and considerable research has been done on it in relation to autism in the past two decades.[4] The gut microbiota is comprised of, viruses, protozoa, and mostly bacteria, the quantity of which exceeds that of the eukaryotic cells in the human body.[5] These microbiota have been seen to play a role in pathophysiology of various diseases including conventional ones such as inflammatory bowel disease, irritable bowel syndrome and obesity, and unconventional ones such as ASDs, Alzheimer's disease, multiple sclerosis and Parkinson's.[6,7] Alterations have been noted in the gut microbiota of patients with autism in comparison to healthy individuals.[8] Whether these alterations are due to the effect of microbiota on the Gut brain axis; or due to the effect of gut brain axis on microbiota, still remains unclear.

Several experiments have been performed to check the effect of microbiota on brain. These include giving antibiotics to modify gut flora, faecal microbial transplantation (FMT), and germ-free (GF) animal models.[8] Research has shown that disturbance of the microbiota-gut-brain axis may lead to behavioural changes in addition to bowel symptoms. The gut microbiota maintains the integrity of the intestinal barrier its maintenance or healing through probiotics results in improvement of behavioural and mood symptoms in mice models.[9] Microbiota may alter chemicals affecting brain function via the hypothalamus–pituitary–adrenal (HPA) axis. ASD may alter the gut microbiota, and these via the HPA axis via secretion of cortisol effect the immune response and hence the gut microbiota composition.[5] One such effect is shown by a study according to which a certain species of Clostridia if present in abundance is responsible for raised dopamine levels in autistic kids. Dopamine excess is known to cause obsessive compulsive, psychotic and other stereotypical behaviours. Dopamine beta hydroxylase that converts dopamine to norepinephrine in serum is deficient in autistic kids as clostridium decreases its activity. Hence, dopamine levels are high and norepinephrine levels low when clostridium levels increase.[10]

Evidence also supports the role of stress in modulating the intestinal microbiota in adults. In adult mice, psychosocial stress reduced the

proportion of Bacteroides but increased the proportion of Clostridia in the cecum.[8]

This link between the GI symptoms and autism led to the idea for this review. What is the association between the gut microbiota and the gut-brain axis in children with autism? This is the question we explore to answer by checking the various commensal levels and identifying various comorbidities and pathologies forming a link between the two. Moreover, we will see which sequencing technique is the most commonly used.

MATERIAL AND METHODS

This review was conducted to find the link between autism spectrum disorder and gut microbiome. The search included all articles written from 2001 onwards as in 2001 Lederberg defined the term microbiota for the first time in a new light. It can be found in the 'History of Medicine' article in a recent Annals of Internal Medicine.[4] Reviewing and reporting was done as per PRISMA protocol.

The MEDLINE and EMBASE databases from 2001 onwards were searched on the PubMed and Ovid platforms. We used free text words and specific key terms (MeSH in MEDLINE). The MeSH words included "autism spectrum disorder" and "gastrointestinal microbiome". The Boolean operator 'and' was used. Prisma flow chart for the systematic review is shown in the Figure-1.

Studies were selected if they were published after 2001. Moreover, observational studies, case control studies, Randomized Control Trials, systematic reviews, studies conducted on humans and free full text articles were included.

Studies were excluded if they were regarding diseases other than ASD, were performed on animals, showed effects of air pollution on microbes, included Mitochondrial research and which did not include ASD. Furthermore, studies not involving microbiome, metabolomics studies, those involving cell lines, with Microbial transfer therapy, narrative reviews and editorials and those not in English language were excluded.

The data was then extracted from the eligible studies by a structured data extraction form. Data was analysed for frequencies and percentages by SPSS version. 20. Graphs were prepared by using Microsoft Excel 2013.

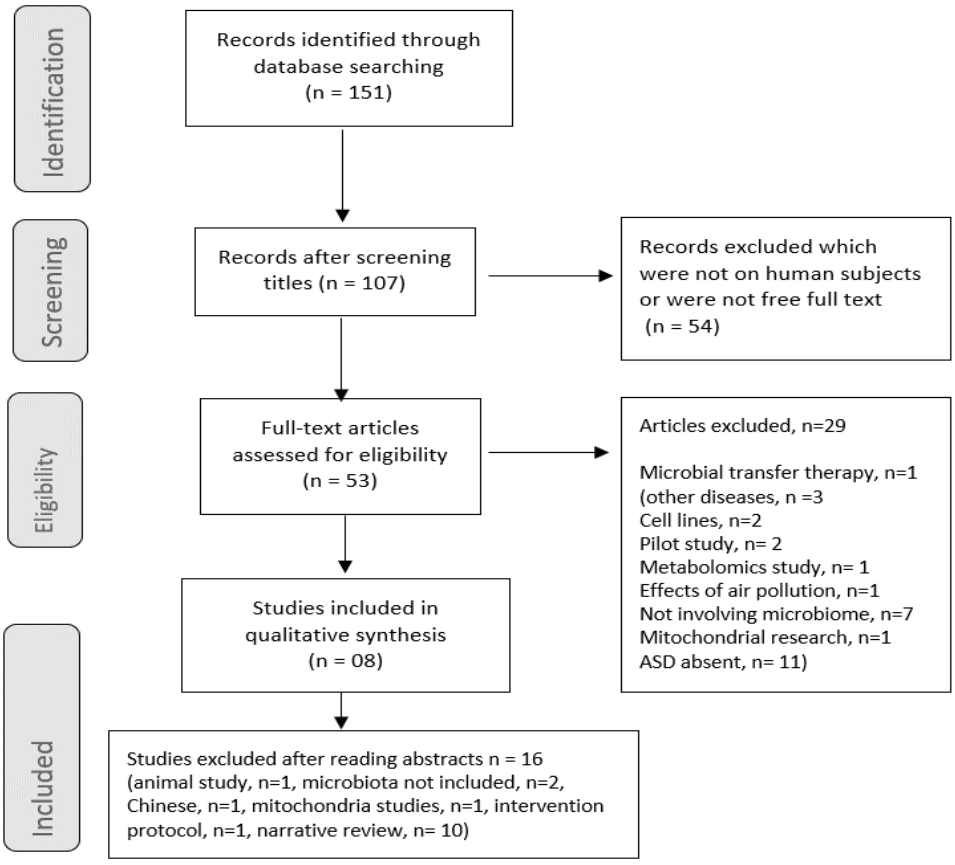

Figure-1: Prisma flow chart showing the selection process

RESULTS

The data search yielded 08 eligible studies (Annexure A) according the inclusion and exclusion criteria previously set. Youngest reported to be of 2 years old and oldest to be 18 years in this review. As for study types 3 were case control, 1 cross sectional, 2 systematic reviews and 2 cohort studies. In majority of studies samples collected were of stool, others included blood, urine and GI biopsy.

Baceroides was seen to decrease in two studies similarly parabacteroides increased in one and decreased in two. Bifidobacterium and Lactobacillus were seen to increase in one study and decrease in two, each. Prevotella was seen to dectrease in three studies and increase in two. A consistent increase of clostridium was seen in four studies. Actinomyces and Proteobacteria were also seen to increase in three studies, each. Bacteroidetes/ Firmicutes ratio was increased in two studies normal in one and decreased in one.

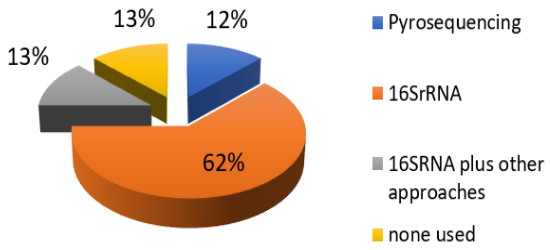

Figure 1 Types of sequencing done in various studies

Table-1: Assessment of methodological quality of included studies child

Assessment of methodology of included studies								
Methods								
First author	**population type (age)**	**study design**	**sampling technique**	**study duration**	**sample size**	**sample type**	**Inclusion criteria**	**Exclusion criteria**
Strati F	Cases age 11.1±6.8 years, controls age 9.2±7.9 years	Cohort study	convenience	not given	40 cases and 40 controls	stool	Autistic kids 11.1±6.8 years and normal kids age 9.2±7.9 years	Autistic subjects with clinically evident inflammatory conditions
Srikantha P	Varied from paper to paper	systematic review	no need	not given	136 studies	GI biopsies, stool, urinary or blood samples	Human studies, ASD individuals, Analysis of bacterial genome by sequencing, peer-reviewed article.	Non full texts, non-English papers, lack of any relevance to the topic.
Liu F	kids 2-18 years	systematic review	no need	not given	17studies	gut biopsy or fecal samples	age 2 to 18, studies with metagenomic sequencing	medicated participants, culture dependent method, intervention studies without initial data or reviews, duplicate publications.
Rose D	children aged 3-12 years	cohort	not given	not given	103 kids	stool and blood	ASD children with GI symptoms of irregular bowel movements, ASD children with no GI symptoms, typically developing children with GI symptoms and typically developing children without GI symptoms.	celiac disease or IBD), use of antibiotics or antifungal medications within the prior month, medications affecting GI transit (stool softeners), and/or recent evidence of a GI infection based on stool laboratory tests, seizure disorder, genetic disorders (i.e. Fragile X syndrome), liver or pancreatic disease, cystic fibrosis, or chronic infection.

Table-2: Assessment of methodological quality of included studies

Assessment of methodology of included studies								
Methods								
First author	**population type (age)**	**study design**	**sampling technique**	**study duration**	**sample size**	**sample type**	**Inclusion criteria**	**Exclusion criteria**
Ma B	cases age 7.04±1.19 controls 7.27±1.07	Case control	not given	December 2015 to july 2017	45 cases and 45 controls	stool	autistic kids 7.04±1.19 normal kids 7.27±1.07	children who had a history of Rett syndrome, cerebral palsy, congenital diseases, and acute/chronic affective diseases in the past 3 months
Zhang M	cases age 4.9±1.5 controls age 4.6±1.1	case control	not given	not given	35 cases and 6 controls	stool	autistic kids age 4.9±1.5 normal kids age 4.6±1.1	Case's children who had fragile X syndrome, tuberous sclerosis, signifcant sensory impairment, clinically evident infammatory conditions, coeliac disease, special diet (such as ketogenic diet) and brain anomalies. Control's children who had psy-chiatric disorders (depressive disorder, schizophrenia and bipolar disorder)
Diaz J.P	Caeses (age in months) 44.51±2.06 Controls 51.00±2.59	Case control	not given	not given	Cases` 48 controls 57	stool	autistic kids (age in months) ANMR 44.51±2.06 AMR 43.69±2.7 normal kids 51.00±2.59	Cases with neurological, metabolic or genetic disorders or on drugs that interfere with results like sedatives/muscle relaxants.
Li N	ASD-Children 59 ages 2–7, Healthy children 30 ages 2–10, ASD-Mothers 59 ages 26–38 Healthy mothers 30 ages 27–42	cross sectional	not given	not given	59 ASD children and their mothers, 30 matched healthy neurotypical children and their mothers	stool	ASD-Children 59 ages 2–7, Healthy children 30 ages 2–10, ASD-Mothers 59 ages 26–38 Healthy mothers 30 ages 27–42	ASD children with inflammatory symptoms.

Table-2: Assessment of changing trends of microbiota in autistic children

Studies	**Bacteria increasing**	**Bacteria decreasing**	**Bacteroidetes/ Firmicutes ratio**
Ma B	*clostridium*	*Acidaminococcaceae, Tyzzerella, Flavonifractor, Lachnospiraceae*	Not mentioned
Zhang M	*Streptococcus, Veillonella and Escherichia*	*Not mentioned*	increased
Diaz J.P	*Actinobacteria, Proteobacteria, Bifidobacteriaceae, Microbacteriaceae, Thermoanaerobacteraceae, Corynebacteriaceae, Clostridiales* and *Thermoactinomycetaceae*	*Not mentioned*	Not mentioned
Li N	*Clostridium, parabacteroides and Streptococcus*	*Prevotella* and *Ruminococcus*	normal
Strati F	*Collinsella, Corynebacterium, Dorea,* and *Lactobacillus*	*Prevotella, Alistipes, Bilophila, Dialister, Parabacteroides*	increased
Srikantha P	*Proteobacteria,Sutterella,Actinomyces, Oscillospira, Peptostreptococcus and Candida.*	*Lactobacillus , Bifidobacterium, Prevotella, Bacteroides, Devosia, Streptococcus*	Not mentioned
Liu F	*Firmicutes Proteobacteria,Prevotella, Veillonella,Clostridium*	*Bacteroidetes, Fusobacteria, Verrucomicrobia, Bifidobacterium, Lactobacillus, Enterobacter* and *Shigella, Akkermansia*	decreased
Rose D	*Bacteriodaceae, Lachnospiraceae, Prevotellaceae* and *Ruminococcaceae.*	Not mentioned	Not mentioned

DISCUSSION

Gut microbiota is the ruling factor when it comes to gastrointestinal health. It also controls the immune responses and the gut-brain axis.[11] In a study with 2973 children with ASD, 24% of the subjects experienced at least one type of GI problem.[12]

Faecal microbiota is a diverse bacterial ecosystem. The bacterial taxonomy constitutes kingdom, phylum, class, order, family, genus and species.[7] The bacterial population of the gut constitutes mainly 4 major phyla which are: Bacteroidetes (such as the Bacteroides and *Prevotella genera*), Firmicutes (such as Lactobacillus, *Clostridium* and *Ruminococcus*), Proteobacteria (such as the Enterobacter species) and Actinobacteria (e.g., *Bifidobacterium).*[13] This ecosystem can be divided into two groups of genera; dominant and subdominant, based on amount of the organisms per gram. The dominant group constitutes *Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus, Ruminococcus, Clostridium and Propionibacterium* with level of $>10^9$ Colony Forming Units (CFU)/g. The subdominant group consists of *Streptococcus, Enterococcus, Lactobacillus, Fusobacterium,Desulfovibrio and Methanobrevibacter* ($< 10^9$ CFU/g).[14]

Firmicutes and Bacteroidetes are the major genera of the bacterial kingdom. The ratio of Bacteroidetes/Firmicutes was observed to be significantly higher in Chinese children with ASD. Whereas in previously conducted studies a decrease was seen in this ratio.[15] The amounts of the so-called harmful and harmless microbes may determine the pathophysiology of a disease.[16] But as it is said "Both scarcity and overabundance tend to produce unhappiness in an individual."[17] Lets take the example of Fermicutes some studies suggest its raised levels may be responsible for obesity and such related aetiologies.[18] Others say its decreased levels may lead to anxiety and major depressive disorder and other neuropsychiatric conditions.[16] In studies lower amounts of the phylum Firmicutes and higher levels of Bacteroidetes have been reported. The metabolites, especially propionic acid, produced by Bacteroidetes may influence the CNS and may induce autism like behaviour by modulating the gut-brain axis.[13] In the present study Bifidobacterium was seen to increase in one study and decrease in two in children with autism. Bifidobacterium has anti-inflammatory properties and hence a protective role in autism, it is also seen to decrease in autism.[19] Similarly Prevotella is also reduced which is necessary of carbohydrate digestion and fermentation.[21]

One of the hypotheses describing the role of gut commensals in autism pathophysiology is, the concept of the leaky gut (abnormal intestinal permeability). That is chemicals produced by, or as a result of presence of microbiota of gut cross the blood brain barrier and effect the brain functions.[21] Dysbiosis also results in over expression of proinflammatory cytokines, such as IL-1β, IL-6, tumour necrosis factor-α.[22] Another mechanism could be that due to abnormal intestinal wall permeability, yeast, toxins, bacteria, viruses and food damage the wall. The body then generates an immune response and produces antibodies against the onslaught. This induced immunity then disrupts the gut-brain axis resulting in not only bowel symptoms but also behavioural issues such as hyperactivity and irritability.[23]

Two popular approaches for researching microbiome are 16Sr RNA (amplicon) and shotgun sequencing (Next Generation Sequencing). 16S rRNA gene sequencing has been the mainstay of research on human gut microbiota, for identifying various genera and species in various phyla. This technique is based on DNA sequence of regions between conserved areas of 16S rRNA which vary among different bacterial species and can be species specific. It amplifies DNA using a set of primers based on the highly-conserved 16S rRNA to sequences of known bacterial taxa in a database.[24]

In comparison Next Generation Sequencing (NGS) which also includes pyrosequencing and shotgun sequencing engages a genome-wide approach, utilizing random strings of genomic DNA sequences obtained by breaking total genomic DNA and matching the obtained sequences to an annotated database of known DNA sequences.25 According to Shashank Gupta bacteria identified by NGS represented 75.70% of the unique bacterial species cultured in each sample, while traditional culture methods only identified 23.86% of the bacterial species found by amplicon sequencing. Yet NGS is being used less commonly in microbiome researches as compared to 16SrRNA sequencing probably because of the cost effectiveness and ease of use of the later.[26]

CONCLUSION

The development of Autism Spectrum Disorder and its manifested symptoms are effected by microbiota of the gut. These symptoms may include social hesitancy, repetitive behaviour, anxiety and depression. Moreover, the excess or dearth of a microbe may lead to deranged pathophysiology so any microbe if not present in suitable amounts may be harmful. Same goes for Bacteroidetes/Firmicutes

ratio. No sequencing technique may be labelled as best, it basically depends upon the objectives of the research.

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