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Isolation And Identification Of Microbiota Species Isolated From Stool Specimens Among Autism Spectrum Disorder Children In Thi-Qar Province, Iraq

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Abstract:

Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder within a numerous neuropsychiatric disorders have been linked to the microbiota.ASD is including anxiety, schizophrenia, autism, bipolar, and depression disorder genetic ingredient that manifests itself in various distinct phenotypes. The term "microbiota" points to the microbial population found within the human body, including bacteria, viruses, archaea, protozoans, and fungi. The combination of bacteria, , and archaea Settle down the gastrointestinal tract is called the 'gut microbiota' and has evolved with the steward over thousands of years to form a complex and exchange advantage relationship.

Method: One hundred autistic patients were enrolled in this study. Fifty healthy individuals were participated in this study. Stool specimens of ASD children were cultured on thioglycolate broth then transfer into selective and differential media (blood agar, Mac Conky agar, EM Bagar, Mannitol salt agar and Chocolate agar) Phenotypic characteristics were identified using API 20 system**.**

Result: In current study, API20 system was used for identification the isolates. The results showed Enterobacteriaceae members more prevalence such as Escherichia coli (13%), Enterobacter spp. (15%), Citrobacterspp (6%), Pantooeaspp, Serratiaodorifera and Kluyvera spp. (2%). Other bacterial that were isolated Enterococcus spp. 3% while Staphlococcusspp (6%),as well as Listeria spp, Micrococcus ,and Neisseria sicca less common.

Conclusion: It is evident that ASD affects the gut microbiota, although additional research is needed to determine if this is a cause or effect of the disorder.

Keywords: Autism, Microbiota, Enterobacteriaceae

Introduction:

ASD is a term that refers to a group of neurodevelopmental disorders marked via genetic and phenotypic diversity among those affected. Affected people with ASD have a mix of abnormalities in adaptive and social functioning, language, and cognitive skills (McPartland and Law,2016; lord,2018). The term "microbiota" points to the microbial population found within the human body, including bacteria, viruses, archaea, protozoans, and fungi (Bakhtiaret al., 2013). The combination of bacteria, eukarya, and archaea Settle down the GI tract is called the 'gut microbiota' and has evolved with the steward over thousands of years to form a complex and exchange advantage relationship(Neish,2009). Researchers found that the GI tract houses an estimated 10^{14} bacteria, which is roughly ten times more than the number of human cells and one hundred times the number of genomic content (microbiota) cells. Its estimates suggest that the approximate ratio of humans to bacteria is one-to-one (Sender et al.,2016). The human gut microbiota, with over 10^3 species and $7*10^3$ strains specified, is a quite complex ecosystem. With a majority of species belonging to the phyla Bacteroidetes (species such as Bacteroides) and Firmicutes (species such as Lactobacillus, Enterococcus, and Clostridium) Additionally, other phyla such as Proteobacteria (Escherichia coli), Actinobacteria (Bifidobacteria), Cyanobacteria, Fusobacteria, and Verrucomicrobia are present in lesser abundance (Qin et al., 2012). The microbiota provides a range of physiological functions, such as protecting the intestinal lining(Natividad and Verdu,2013), providing energy and nutrients and shelter, and shaping the tissue(den Bestenet al.,2013). It can digest food, including metabolizing the byproducts, all of enhancing the host's overall health(Bäumler and Sperandio, 2016). Numerous neuropsychiatric disorders have been linked to the microbiota, including anxiety, schizophrenia, autism, bipolar, and depression disorder (Inserm, 2018).Abbaset al. (2020)showedthe altered gut microflora might play an essential role in the pathogenesis of autism. The main aim of the study is to detect microbiota species from stool specimens among Autism spectrum disorder children.

Material and method:

One hundred autistic patients were enrolled in this study. Fifty healthy individuals were participated in this study. Stool specimens of ASD children were cultured on thioglycolate broth then transfer into selective and differential media(blood agar, Mac Conky agar, EMB agar, Mannitol salt agar and Chocolate agar). Phenotypic characteristics were identified with API20 system. Ethical management of the study was approved according to recommendations guide gained from the College of Science, University of Thi-Qar. The study did not include forbidden biological materials or genetically modified organisms. All stool specimens that included in this study were obtained from patients Thi-Qar Autistic Disorders Rehabilitation Center after obtaining official approvals by the doctor and the patient who allowed the collection of samples.

Result:

In the current study, API20 system was used for each of the Staphylococci, Streptococci and Enterobacteriaceae. The results showed Enterobacteriaceae members more prevalence such as E.coli(13%),Enterobacter spp. (15%), Citrobacter spp.(6%),Pantooeaspp., Serratiaodorifera and Kluyvera spp.(2%). Other bacterial isolates were Enterococcus spp.(3%) while Staph.spp.(6%),as well as Listeria spp, Micrococcus, and Neisseria sicca less common. The results were showed in Table (1).

Table (1) Distribution of microbiota in causes of ASD and control

Type of bacteria	ASD No.=100		Control No.=50		X^2	P value
	No.	%	No.	%		
E. coli	13	13	3	6	1.71	0.190*
E. coli+ Enterobacter	3	3	4	8	1.87	0.171*
E. coli + Staph. lentus	4	4	0	0	2.05	0.152*
E. coli + lecuonstoc	2	2	0	0	1.014	0.314*
E. coli + Citrobacter	3	3	0	0	1.53	0.216*
E. coli ∕ Kluyvera spp.	2	2	0	0	1.014	0.314*
Citrobacter spp.	6	6	1	2	1.19	0.274*
Enterobacter spp.	15	15	0	0	8.33	0.004**
Enterococcus spp.	3	3	0	0	1.53	0.216*
Staph. spp.	6	6	1	2	1.19	0.274*
Pantooea spp.	2	2	0	0	1.014	0.314*
Serratiaodorifera	2	2	1	2	0	1*
Kluyvera spp.	2	2	0	0	1.014	0.314*
Listeria spp.∕Micrococcus	2	2	0	0	1.014	0.314*
Neisseria sicca	2	2	0	0	1.014	0.314*
Strept. Agalactiae ∕Citrobacterkos.	3	3	0	0	1.53	0.216*
No growth	30	30	40	80	33.48	0**
Total	100	100	50	100		
X^2	154.93		534.9			
P value	0**		0**			

No significant difference at P<0.05, **Significant difference at P<0.05

Dissection

Previous research has established that genetic and epigenetic factors are critical underlying factors in autism spectrum disorders. Similarly, over the last two decades, significant research has been conducted on the role of gut microbiota (GMB) in the severity of autism spectrum disorder (ASD). GMB is a collection of microorganisms, primarily bacteria, which that reside primarily on the external and internal surfaces of the digestive tract, skin, and oral mucosa (Madison and Kiecolt-Glaser,2019). GMB communicates with the brain via the microbiome-gut-brain axis, modulating brain development, immunity, and metabolic homeostasis in the process. Disrupting this communication can result in a variety of disorders, including autism spectrum disorder (Hajjo and Geva-Zatorsky, 2020).

The current study was showed the different compositions of microbiota bacterial species isolated including E.coli only 13%, E.coli with other bacteria from same patient 14% comparison with other study showed high level E. coli in ASD children (Strati et al., 2017). Another study showed E. coli is less abundant (Zhang et al. ,2018).Whilst Neisseria sicca in the study showed 2% approved by previous study Neisseria less abundant in ASD (Kushak et al., 2017).

Thepresent study showed other Enterobacteraceae genus isolated including Enterobacter spp.(18%), Citrobacter spp.(12%), Klyveriaspp. (4%), Pantooea spp.(2%)and Serratiaodorufera (2%) Compared to previous research high Enterobacteraceae prevalence (Plaza-Diaz et al. , 2019).Streptococcus agalactiae also isolated in current study (3%),Streptococcus in previous study showed increasing in ASD children (Li et al., 2019). As well Listeria spp. 2% isolated in present study compared with Coretti et al.,(2018), Firmicutes higher in ASD child. Microccocus spp.(2%) isolated other study showed while previous study was showed reduction of Actinobacteria (Corettiet al., 2018).Enterococcus (3%) showed in current study compared with previous study done by Plaza-Diaz et al.,(2019) which high level in ASD.

Staphlocco cuslentus and Staphylococcus spp. showed(4%),(6%) previous study showed decrease in firmicutes in ASD (Tomova et al., 2015).In present study isolated leconustic(2%)not compatible with previous research clarified increase by Lactobacillusspp.(Strati et al. ,2017).

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