Journal of Molecular Neuroscience (2021) 71:153–161
https://doi.org/10.1007/s12031-020-01636-2

Sensory Processing and Gastrointestinal Manifestations in Autism Spectrum Disorders: No Relation to *Clostridium difficile*

Mona Khalil[1] · Hanan Galal Azouz[1] · Shwikar AbdelSalam Ahmed[2] · Hala Ali Gad[1] · Omneya Magdy Omar[1]

Received: 3 April 2020 / Accepted: 12 June 2020 / Published online: 30 June 2020

Abstract

The role of the gut microbiota in triggering autism is a rapidly emerging field of research. Gut microbiota have been incriminated because autistic children often have gastrointestinal symptoms. Pathogenic gut bacteria in children with autism spectrum disorders (ASD) have been reported. The present study aimed to assess *Clostridium difficile* in the stool of children with ASD and its relation to gastrointestinal (GI) comorbidities, autism severity, and sensory impairment. The study included 58 ASD patients, 45 of their neurotypical siblings, and 45 unrelated controls. Childhood Autism Rating Scale (CARS) was used to assess the severity of autism. Sensory problems were evaluated using the Short Sensory Profile (SSP). GI symptoms were assessed with a modified six-item GI Severity Index (6-GSI) questionnaire. Quantitative real-time PCR was done for the detection and quantitation of *C. difficile* and its toxins A and B. *C. difficile* was detected in 25.9%, 40%, and 15.6% of ASD cases, siblings, and unrelated control respectively. Regarding toxin A and B production, 73.3%, 77.8%, and 71.4% of *C. difficile* in positive ASD, siblings, and unrelated control cases respectively were toxigenic. There was no statistically significant difference between the three groups as regards *C. difficile* qualitative, quantitative, and toxin production results. In conclusion, *C. difficile* is not specifically prevalent in the gut of children with ASD. Although most of the strains are toxigenic, there were no GI symptoms in the control groups and no statistically significant association with GI Severity Index in autistic cases. Gastrointestinal dysfunction and sensory impairment are common comorbidities in ASD.

Keywords Autism · *Clostridium difficile* · Gastrointestinal symptoms · Short Sensory Profile

Introduction

Autism spectrum disorders are neurodevelopmental disorders that comprise autism, Asperger's syndrome, and pervasive developmental disorder not otherwise specified (PDD-NOS). It has many consequences in cognitive and sociability impairments, impairments, impairments in language and communication skills, restricted interests, and stereotyped behaviors (Johnson and Myers 2007).

A diagnosis of ASD is typically made before the age of 3 years, and the diagnoses have dramatically increased in the past years; ASD are about four times more prevalent in males than females, for unclear reasons (Centers for Disease Control and Prevention (CDC) 2014.

While the etiology of ASD remains unidentified, evolving evidence suggests multiple gene defects may be involved in tandem with an environmental catalyst (Cusco et al. 2009; Heberling et al. 2013). Gut microbiota have been incriminated because children with ASD often have gastrointestinal problems that correlate with ASD severity (Wang et al. 2011). Numerous previous studies have stated pathogenic gut bacteria in children with ASD (Li et al. 2017; Rosenfeld 2015; Wang et al. 2013).

Clostridium difficile may be present as a colonizing inhabitant of the normal gut microbiota of some individuals, however, provoke no visible signs of disease. Most cases of *C. difficile* infection appear in patients who are prescribed high-dose or long-course antibiotics, which disrupt the normal balance of the gut microbiota, changing its composition and leading to the overgrowth of *C. difficile* bacteria (Van den Abbeele et al. 2013).

The *Clostridium* hypothesis started by Sandler et al. (2000), in which children with regressive autism were treated

✉ Omneya Magdy Omar
O_magdy09@alexmed.edu.eg

[1] Department of Pediatrics, Faculty of Medicine, Alexandria University, Alexandria 21321, Egypt

[2] Department of Medical Microbiology and Immunology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

with oral vancomycin for 6 weeks. Noteworthy improvement in GI and neurobehavioral symptoms was noticed in eight of the ten children, providing suggestion in favor of a toxin-producing *Clostridium* as a possible reason of regressive autism. However, gradual regression in bowel and behavioral symptoms occurred in all subjects following the discontinuation of vancomycin. This relapse was explained by the fact that many *Clostridia* can transform into spore-form, which are greatly resistant to antibiotics, but can then later germinate into vegetative, infective forms, and continue toxin production. Moreover, the fact that oral vancomycin's effects are confined to the intestinal tract and not systemically absorbed provides further support for the involvement of the intestinal microbiota (Sandler et al. 2000).

Some studies endorse the *Clostridium* hypothesis, although the accurate species responsible have not been fully clarified (Finegold et al. 2002; Song et al. 2004).

In the present study; we aimed to assess *Clostridium difficile* in the stool of children with ASD and its relation to GI comorbidities, autism severity, and sensory impairment in our population.

Subjects and Methods

Fifty-eight autistic children, who presented to the Autism Clinic of Alexandria University Children's Hospital, were enrolled in our study. These children were diagnosed with ASD stated by the diagnostic and statistical manual of mental disorders fifth edition (DSM-5) criteria (American Psychiatric Association (APA 2013). Their age ranged from 3 to 10 years. Forty- five neurotypical siblings and a cross- matching unrelated control group of 45 normally developing children of similar age and sex were also included.

Children with ASD with known syndromes, hepatic impairment, or immune deficiencies were excluded from the study.

Approval of the ethics committee of the Faculty of Medicine, Alexandria University, was obtained. Parents were explained about the study and written informed consent was obtained for their children's examination and intervention.

All the studied children were subjected to thorough history taking and complete physical examination with special emphasis on neurological examination. The severity of autism was assessed by using Childhood Autism Rating Scale (CARS) (Rellini et al. 2004).

Presence or absence of sensory problems was assessed in children with ASD using short sensory profile (SSP) (Dunn 1999). The SSP is a 38-item caregiver report. Items were scored on a 1-point to a 5-point scale. The seven sections of the SSP were tactile sensitivity, taste/smell sensitivity, movement sensitivity, under responsive/ seek sensation, auditory filtering, low energy/weak, and visual/auditory sensitivity. Cases were classified as having definite sensory impairment, probable sensory impairment, or typical performance for each of these sections and a total score. Both section scores and a total score were interpreted on the SSP and were treated as the independent variables. The total score is the most sensitive indicator of sensory dysfunction.

Gastrointestinal (GI) symptoms were assessed using a modified short version of the GI Severity Index; 6-GSI questionnaire (Schneider et al. 2006). It consisted of six items (constipation, diarrhea, stool consistency, stool smell, flatulence, and abdominal pain). Every variant took a score of 0, 1, or 2 according to its frequency per week; zero scores were interpreted as the absence of the symptom while 1 and 2 denoted presence of the symptom with different severity. A total score equal or less than three was classified as low score and more than three was a high score.

C. difficile Detection

Specimen Collection, Preservation, and Transport

Stool specimens were collected, kept in the freezer upon defecation at home, and within the same day delivered to our laboratory frozen, where aliquots of each specimen were frozen at − 80 °C until DNA extraction in the same week.

DNA Extraction

DNA was extracted from 150-mg stool samples using ISOLATE Fecal DNA Kit (Bioline, UK) according to the manufacturers' information. Fecal samples were added directly to a bashing beads lysis tube and they were rapidly lysed by bead beating in a vortex, without the use of organic denaturants or proteinases. The DNA was then bound, isolated, and purified using spin columns. The resulting DNA extracts were stored at − 80 °C until PCR assessment.

Detection and Quantitation of *C. difficile*

All the sequences of the primers were obtained from the previously published studies. Oligonucleotide primers targeted the 16S rRNA gene (rDNA) sequences of *C. difficile* were used (Penders et al. 2005). Primers were also used to amplify a conserved 16S rDNA sequence present in all bacteria (universal primer set, recognizing domain bacteria), and the amplification of which served as the denominator against which the amplification of the *C. difficile* was compared (Nadkarni et al. 2002). Primers were commercially obtained (Metabion International AG, Germany).

Amplification was performed in a light cycler (Rotor Gene Q, Qiagen, Germany) using a SensiFAST ™ SYBR No-ROX PCR kit (Bioline Co. UK). In brief, forward and reverse primers (4 pmol each) were used in 20 μl reactions containing 2 μl of the DNA extract.

PCR amplification was achieved with an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C. Melting curve analysis was achieved from 40 to 95 °C with a plate reading step after every 1 °C and held at a particular temperature for 10 s to check the specificity of the product formed.

Quantitation of specific bacteria DNA is not expressed as an absolute number but expressed relative to total bacterial DNA present in the stool sample. Mean of relative abundance value of the bacteria is shown in instances in which the decimal value is low, as E−05 (4.75×10^{-5} is shown as 4.75E−05) (Balamurugan et al. 2008).

Detection of *C. difficile* Toxins A and B

This assay is based on the amplification of the genes encoding toxins A and B, the major virulence factors of this bacterial species. Amplification was accomplished by utilize 0.7 μM (each) primers tcdA442 and tcdA579, and 0.45 μM (each) primers tcdB2667 and tcdB2746 each in a separate reaction tube.

PCR amplification was performed with a primary denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 57 °C for 15 s, and extension at 72 °C for 7 s. Melting curve analysis was performed from 40 to 95 °C with a plate reading step after every 1 °C and held at a particular temperature for 10 s to check the specificity of the product formed (Belanger et al. 2003).

Statistical Analysis of the Data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using the number and percent. The Kolmogorov-Smirnov test was used to confirm the normality of distribution. Comparisons between groups for categorical variables were assessed using chi-square test (Fisher or Monte Carlo). The Student *t* test was used to compare two groups for normally distributed quantitative variables. Kruskal-Wallis test was used to compare different groups for abnormally distributed quantitative variables and the Mann-Whitney test was used to compare between two groups. Spearman coefficient was used to correlate between quantitative variables. Significance of the obtained results was judged at the 5% level.

Results

Out of the 58 ASD children, 39 (67.2%) were males and 19 (32.8%) were females with male to female ratio of 2.1:1. Their age ranged from 3 to 10 years with a mean of 5.41 ± 1.55 years. Out of the 45 siblings, 22 (48.9%) were males and 23 (51.1%) were females. The mean age was 4.31 ± 3.23 years and ranged from 0.5 to 12 years. For the unrelated control, out of 45 controls, 28 (62.2%) were males and 17 (37.8%) were females. The mean age was 5.36 ± 2.6 years and ranged from 2 to 12 years.

According to CARS, 46 (79.3%) of ASD patients were mild to moderate (CARS < 36), while the other 12 (20.7%) were severe ASD (CARS ≥ 36). The CARS range was 30–45 with mean 33.2.

Regarding the GI symptoms at the time of examination, all ASD cases had at least one GI symptom. The most frequent symptom was offensive stool odor (86.2%), and the least was diarrhea (12.1%). The mean of the Total GSI score was 3.95 ± 1.58; low in 19 (32.8%) cases and high in 39 (67.2%) cases. (Table 1).

Table 2 demonstrates the SSP score in children with ASD. The highest definite abnormality detected in 91.4% of cases was in the under responsive/seeks sensation, while the lowest was in the visual/auditory sensitivity which was found in only

Table 1 Distribution of children with ASD according to GSI score ($n = 58$)

Symptom score	*n*	%
Constipation		
0	25	43.1
1	17	29.3
2	16	27.6
Diarrhea		
0	51	87.9
1	4	6.9
2	3	5.2
Stool consistency		
0	46	79.3
1	11	19.0
2	1	1.7
Stool smell		
0	8	13.8
1	16	27.6
2	34	58.6
Flatulence		
0	18	31.0
1	31	53.4
2	9	15.5
Abdominal pain		
0	37	63.8
1	18	31.0
2	3	5.2
Total score		
Low ≤ 3	19	32.8
High > 3	39	67.2

8.6% of children with ASD. The total score of SSP among the children with ASD showed definite impairment in 52 (89.7%) cases, probable impairment in 6 (10.3%) cases, and no one had the typical performance on the total score.

Table 3 illustrates the correlation between CARS, 6-GSI, and SSP among children with ASD and shows that there was a significant positive correlation between CARS and 6-GSI. Also, there was a significant negative correlation between CARS and total score of SSP. However, there was no statistically significant correlation between 6-GSI and total score of SSP.

Clostridium difficile was detected in 15 out of 58 (25.9%) ASD cases, 18 (40%) and 7 (15.6%) out of 45 siblings and 45 unrelated control respectively. As regards the relative abundance of *C. difficile* in positive cases, the median was 6.98E−06, 4.19E−06, and 5.71E−06 among the ASD, siblings, and unrelated control respectively. Although the percentage of *Clostridium difficile* was higher in ASD cases and their siblings than the unrelated control, there was no statistically significant difference between the three groups either qualitative or quantitative results (Tables 4 and 5).

Regarding toxin production, *C. difficile* was classified as toxin A and/or B producers or non-toxigenic. Table 6 shows that 11 (73.3%), 14 (77.8%), and 5/7 (71.4%) of *C. difficile* in positive ASD, siblings, and unrelated control cases respectively are toxigenic. Among the positive ASD cases, there are no bacteria produce A and B toxins together; A−B+ producers are found in 4 (26.7%), A+B− were found in 7 (46.6%), and non-toxigenic (A−B−) were found in 4 (26.7%) patients. In siblings, there are 1 (5.6%) A+B+ producers, 4 (22.2%) A−B+ producers, 9 (50%) A+B− producers, and 4 (22.2%) non-toxigenic strains. In unrelated controls, there are no one produce A+B+, 2 (28.6%) A−B+ producers, 3 (42.9%) A+B− producers, and 2 (28.6%) non-toxigenic strains. Also, there was no statistically significant difference between the three groups ($p = 0.232$) (Table 6).

Studying the relation between the presence or absence of *C. difficile* in children with ASD with CARS and SSP and 6-GSI score revealed that there was no statistically significant difference between negative and positive cases for *C. difficile* and CARS or SSP or 6-GSI score (Table 7).

Table 2 Distribution of children with ASD according to SSP score ($n = 58$)

Item	*n*	%
Tactile sensitivity		
Definite	49	84.5
Probable	4	6.9
Typical	5	8.6
Taste/smell sensitivity		
Definite	25	43.1
Probable	14	24.1
Typical	19	32.8
Movement sensitivity		
Definite	10	17.2
Probable	7	12.1
Typical	41	70.7
Under responsive/seeks sensation		
Definite	53	91.4
Probable	4	6.9
Typical	1	1.7
Auditory filtering		
Definite	40	69.0
Probable	11	19.0
Typical	7	12.1
Low energy/weak		
Definite	17	29.3
Probable	7	12.1
Typical	34	58.6
Visual/auditory sensitivity		
Definite	5	8.6
Probable	9	15.5
Typical	44	75.9
SSP total score		
Definite	52	89.7
Probable	6	10.3
Typical	0	0.0

Discussion

Gut microbiota possess potential involvement in a range of neurodevelopmental disorders as well as its impact on behavior and mood (Diaz Heijtz et al. 2011). Oral vancomycin treatment has been also reported to temporarily reduce autism-associated behavioral abnormalities, although this study did not depend on microbiological detection of specific bacteria (Sandler et al. 2000).

In the present study using quantitative real-time PCR, 15 (25.9%) in ASD group were positive for *C. difficile* compared with 18 (40%) in siblings and 7 (15.6%) in the unrelated control group, although higher in ASD and their siblings, the

Table 3 Correlation between different parameters in autism group ($n = 58$)

	r_s	*p*
CARS vs. GI Severity Index	0.432*	0.001*
CARS vs. SSP total score/190	− 0.433*	0.001*
GI Severity Index vs. SSP total score/190	− 0.234	0.077

r_s Spearman coefficient

*Statistically significant at $p \leq 0.05$

Table 4 Comparison between the studied groups according to qualitative results of *C. difficile*

C. difficile	Autism ($n = 58$) (%)	Normal sibling ($n = 45$) (%)	Control ($n = 45$) (%)	Test of significance	p value
Positive	15 (25.9)	18 (40)	7 (15.6)	$\chi^2 = 5.737$	0.057
Negative	43 (74.1)	27 (60)	38 (84.4)		

Qualitative data were described using number and percent

χ^2, p χ^2 and p values for chi-square test for comparing between the studied groups

results were statistically insignificant. Moreover, there was no statistically significant difference between the studied groups according to toxin A and/or B production. Similar findings were detected in other studies.

Finegold et al. (2010) investigated GI microbiota in 33 autistic children, 7 neurotypical siblings, and 8 unrelated controls by using pyrosequencing. They did not find Clostridia to be specifically prevalent in the stools of autistic individuals (Finegold et al. 2010). Similarly, Martirosian et al. (2011) in a study to assess the presence of *C. difficile* toxins, included 41 autistic children and 10 healthy children, isolated *Clostridium* spp. strains were subjected to sequencing in order to search for *C. histolyticum* and it showed negative results (Martirosian et al. 2011). Also, Gondalia et al. found that there was no significant difference in the bacterial composition of fecal material of the autistic group and their typically developing siblings (Gondalia et al. 2012).

In Egypt, the gut microbiome in autism is not well studied, and even *C. difficile*-associated diarrhea (CDAD) and asymptomatic carriage are still not well estimated especially in pediatric patients because the anaerobic of stool samples is not a routine in our country and to some extent is considered expensive.

Abd El-Wahab et al. (2016), in Egypt, conducted a study to determine the incidence of *C. difficile* as an etiology of antibiotic-associated diarrhea in 60 hospitalized children with a history of antibiotic intake. Stool samples were processed for *C. difficile* isolation and examined for *C. difficile* toxins A and B by the enzyme-linked immunosorbent assay. The reported CDAD in their hospital was 46.2%. The asymptomatic carriage rate with toxigenic *C. difficile* was 11.8%. No one of the control infants and children had diarrhea and all of them were negative for *C. difficile* organism and toxin which is much less than our results which may be attributed to using different methods for detection (Abd El-Wahab et al. 2016).

In contrast, Parracho et al., using fluorescence in situ hybridization (FISH) targeting *Clostridium* groups, reported that levels of the *Clostridium histolyticum* group of bacteria were higher in the ASD children compared with typical children. Also, there was no significant difference detected in this bacterial population (*Clostridium* clusters I and II) between the ASD children and their healthy siblings, but total *Clostridium* was higher in ASD group than sibling group (Parracho et al. 2005).

Luna et al. found a significant increase in several mucosa-associated *Clostridiales* detected in ASD children with functional gastrointestinal disorders. They took a rectal biopsy to detect Clostridiales (Luna et al. 2017).

This difference between the current study and previously mentioned studies could be explained by different techniques, different environment, small studied sample, or different samples.

In the current study, all cases had at least one GI symptom; the most frequent symptom was offensive stool odor and the least was diarrhea. In previous researches, the results are variable. The reported prevalence of GI symptoms in children with ASD has ranged from 9 to 70% or higher (Black et al. 2002; Horvath and Perman 2002).

A study done by Parracho et al. found a high proportion of ASD patients had GI disorders (91.4%). Diarrhea was the most common GI symptom (75.6%), followed by excess wind (55.2%), abdominal pain (46.6%), constipation (44.8%), and abnormal feces (43%). Some autistic individuals were noted to suffer from various GI problems, including both diarrhea and constipation (Parracho et al. 2005).

In another study by Wang et al., parents reported significantly more GI problems in children with ASD (42%). The two most common Gl problems in children with ASD were constipation (20%) and chronic diarrhea (19%). Increased autism symptom severity was associated with a higher score of

Table 5 Comparison between the studied groups according to quantitative results *of C. difficile*

C. difficile positive	Autism ($n = 15$)	Sibling ($n = 18$)	Control ($n = 7$)	Statistical test	p
Mean ± SD	2.56E−05	6.73E−06	1.39E−05	$H = 2.27$	0.321
Median	6.98E−06	4.19E-06	5.71E-06		
Min.–Max.	1.56E−06–2.03E−04	5.04E−07–3.81E−05	5.80E−07–5.17E−05		

H, p H and p values for Kruskal-Wallis test

Table 6 Comparison between the studied groups according to *C. difficile* toxin production

C. difficile positive Toxin	Autism ($n = 15$) No.	Sibling ($n = 18$) %	Control ($n = 7$) No.	χ^2	p
Non-toxin producer					
A−B−	4	26.7	4		
Toxin producer				10.085	0.232
A+B+	0	0.0	1		
A−B+	4	26. 7	4		
A+B−	7	46. 7	9		
Total	11	73.3	14		

χ^2, p χ^2 and p values for chi-square test for comparing between the studied groups

GI problems (Wang et al. 2011). Moreover, Garrindo et al. found that functional constipation was the most frequent type of GI diseases in children with ASD (85%) (Gorrindo et al. 2012).

Wasilewska and Klukowski reported that the most common GI symptoms included overproduction of intestinal gasses/flatulence (60%), bloating (38%), abdominal pain (37.8%), diarrhea (28%), burping/belching (25%), gastroesophageal reflux

Table 7 Comparison between children with negative and positive *C. difficile* regarding CARS, SSP, and GI symptoms and GSI score in ASD group

Clinical data	*C. difficile*		Test of significance	p
	Negative ($n = 43$)	Positive ($n = 15$)		
CARS				
Min.–Max. Mean ± SD	28.0–45.0 33.33 ± 4.61	28.0–41.0 32.80 ± 3.80	$t = 0.396$	0.693
Mild to moderate < 36 Severe ≥ 36	34 (79.1) 9 (20.9)	12 (80) 3 (20)	$\chi^2 = 0.006$	1.000
Tactile	22.37 ± 4.19	23.47 ± 4.41	$t = 0.86$	0.393
Taste/smell	12.51 ± 4.48	13.0 ± 3.70	$t = 0.379$	0.706
Movement	12.95 ± 3.32	14.13 ± 2.29	$t = 1.515$	0.139
Under responsive	16.14 ± 4.62	17.13 ± 4.78	$t = 0.711$	0.48
Auditory	17.44 ± 3.89	18.40 ± 3.50	$t = 0.842$	0.403
Low energy	25.95 ± 5.93	24.73 ± 5.18	$t = 0.707$	0.482
Visual	20.79 ± 3.19	19.87 ± 2.88	$t = 0.99$	0.327
Total SSP	128.16 ± 12.04	130.73 ± 10.17	$t = 0.803$	0.429
Constipation, *n* (%)	26 (60.5)	7 (46.7)	$\chi^2 = 863$	0.353
Diarrhea, *n* (%)	5 (11.5)	2 (13.3)	$\chi^2 = 0.030$	1.000
Stool consistency, *n* (%)	7 (16.3)	5 (33.3)	$\chi^2 = 1.971$	0.265
Stool smell, *n* (%)	38 (88.3)	12 (80)	$\chi^2 = 0.656$	0.414
Flatulence, *n* (%)	32 (74.4)	8 (53.3)	$\chi^2 = 2.310$	0.194
Abdominal pain, *n* (%)	18 (41.9)	3 (20)	$\chi^2 = 2.301$	0.129
GSI total score/12				
Min.–Max.	1.0–6.0	0.0–9.0	MW = 1.024	0.306
Median	1.0–6.0	0.0–9.0		
Low GSI ≤ 3, *n* (%)	12 (27.9)	7 (46.7)	$\chi^2 = 1.777$	0.213
High GSI > 3, *n* (%)	31 (72.1)	8 (53.3)		

Qualitative data were described using number and percent, while abnormally distributed data was expressed in median (Min.–Max.)

t for Student's *t* test

χ^2 chi-square test

MW Mann-Whitney *U* test

symptoms (16%), and constipation (10%) (Wasilewska and Klukowski 2015).

In the present study, according to the 6-GSI in the ASD group, the total score was high (3.95), with 67.2% of cases had a high score and only 32.8% cases with a low score. There was no significant difference between positive and negative cases for *C. difficile* as regards the 6-GSI score. However, there was a positive correlation between CARS and GI symptoms.

This agrees with Adams et al. (2011), where the autism group was divided into two groups: low GI problems and high GI problems. The two groups were compared for gut bacteria and there were no significant differences. However, they stated that the strong correlation of the 6-GSI and the ATEC (Autism Treatment Evaluation Checklist) greatly demonstrates that gastrointestinal problems are associated with autism severity. The autism group with high GI problems had significantly higher scores on the ATEC versus the autism group with low GI problems (Adams et al. 2011).

In ASD children, the elevated rates of GI dysfunction might be due to influences other than GI microbiota. For example, it is well recognized that individuals with ASD have higher levels of distress and anxiety and stress activates the hypothalamic-pituitary-adrenal axis, resulting in the neuronal release of catecholamines; activating the sympathetic nervous system could affect the gut mucosa and the sympathetic efferents can impede gut motility proposing a mechanism for constipation (Drahota et al. 2011; Mazefsky et al. 2014).

According to the sensory evaluation in ASD group in the current study, the total sensory checklist score was 52(89.7%) with definite impairment and 6 (10.3%) with a probable difference, and no one met typical performance. The highest definite impairment included under responsiveness/seeks sensation (91.4%).

Similarly, sensory processing sections of the SSP done by Tomchek and Dunn (2007) reported clear differences in the ASD group in the underresponsive/seeks sensation (86.1%), auditory filtering (77.6%), tactile sensitivity (60.9%), and taste and smell sensitivity (54.1%). Other SSP sections had relatively lower percentages of described sensory processing differences in the definite difference range but still a greatly higher percentage than the typically developing group. Particularly, when probable and definite differences classifications were summed as an indicator of some degree of sensory processing differences, 95% of the sample of children with ASD was rated as having some degree of difference in sensory processing based on the SSP total score (Tomchek and Dunn 2007).

Leekam et al. reported that children with autism were more affected by sensory abnormalities than were children with language impairment and developmental disability. Two out of 33 children with autism (6%) were not affected by a sensory symptom, several symptoms in both the visual and in the auditory domains that appeared in 50% or more of the autism (Leekam et al. 2007).

In the current study, there was no relation between *C. difficile* and sensory impairment. However, there was a correlation between autism severity (CARS) and sensory impairment.

Gondalia et al. detected no significant differences within the ASD group when comparing those with and without GI dysfunction as regards the symptoms and the behavior (Gondalia et al. 2012). Moreover, Mazefsky et al. (2014) found that children with and without gastrointestinal problems did not vary in autism symptom severity, adaptive behavior scores, or mean social problem scores.

Conclusions

To summarize, *Clostridium difficile* is not specifically prevalent in the gut of children with ASD. Although most of the strains are toxigenic, there were no GI symptoms in the control groups and no statistically significant correlation with GI Severity Index in autistic cases. Gastrointestinal dysfunction and sensory impairment are common comorbidities in ASD.

Acknowledgments The authors thank the staff members of Pediatric Neurology and Medical Microbiology Department in addition to the participants who provided samples and data for this work.

Author Contributions

- Study concept and design: Hanan, Shwikar Mona, Omneya
- Acquisition of data: Hala.
- Bacteriological analysis: Shwikar.
- Analysis and interpretation of data: Hanan, Shwikar, Mona, Omneya.
- Drafting of the manuscript: Shwikar, Omneya, Hanan, Mona, Hala.
- Critical revision of the manuscript for important intellectual content: Shwikar, Omneya, Hanan, Mona.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Consent to Participate Written informed consent was obtained from the parents.

Ethical Approval All procedures completed in the study involving human participants were in agreement with the ethical standards of the institutional research committee (Medical Research Ethics Committee of Alexandria Faculty of Medicine, Egypt) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

Abd El-Wahab MAA, Naeem AM, Abd E-R, Mohamed E-M, Sallam NRM (2016) Antibiotic associated diarrhea and incidence of Clostridium difficile infection and colonization in infants and

children in Tanta University Hospital. Egypt Int J Curr Microbiol App Sci 5:575–585

Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA (2011) Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. BMC Gastroenterol 11:22. https://doi.org/10.1186/1471-230x-11-22

American Psychiatric Association (APA) (2013) Diagnostic and statistical manual of mental disorders, 5th edn. APA, Arlington, p 501

Balamurugan R, Janardhan HP, George S, Raghava MV, Muliyil J, Ramakrishna BS (2008) Molecular studies of fecal anaerobic commensal bacteria in acute diarrhea in children. J Pediatr Gastroenterol Nutr 46:514–519. https://doi.org/10.1097/MPG.0b013e31815ce599

Belanger SD, Boissinot M, Clairoux N, Picard FJ, Bergeron MG (2003) Rapid detection of *Clostridium difficile* in feces by real-time PCR. J Clin Microbiol 41:730–734

Black C, Kaye JA, Jick H (2002) Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database. BMJ (Clin Res Ed) 325:419–421

Centers for Disease Control and Prevention (CDC) (2014) Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010 Morbidity and mortality weekly report Surveillance summaries (Washington, DC : 2002) 63:1–21

Cusco I et al (2009) Autism-specific copy number variants further implicate the phosphatidylinositol signaling pathway and the glutamatergic synapse in the etiology of the disorder. Hum Mol Genet 18:1795–1804. https://doi.org/10.1093/hmg/ddp092

Diaz Heijtz R et al (2011) Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 108:3047–3052. https://doi.org/10.1073/pnas.1010529108

Drahota A, Wood JJ, Sze KM, Van Dyke M (2011) Effects of cognitive behavioral therapy on daily living skills in children with high-functioning autism and concurrent anxiety disorders. J Autism Dev Disord 41:257–265. https://doi.org/10.1007/s10803-010-1037-4

Dunn W (1999) Sensory profile user's manual. Psychological Corporation, San Antonio

Finegold SM et al (2002) Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 35:S6–s16. https://doi.org/10.1086/341914

Finegold SM et al (2010) Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 16:444–453. https://doi.org/10.1016/j.anaerobe.2010.06.008

Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW (2012) Molecular characterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. Autism Res 5:419–427. https://doi.org/10.1002/aur.1253

Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG, Levitt P (2012) Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. Autism Res 5:101–108. https://doi.org/10.1002/aur.237

Heberling CA, Dhurjati PS, Sasser M (2013) Hypothesis for a systems connectivity model of autism spectrum disorder pathogenesis: links to gut bacteria, oxidative stress, and intestinal permeability. Med Hypotheses 80:264–270. https://doi.org/10.1016/j.mehy.2012.11.044

Horvath K, Perman JA (2002) Autism and gastrointestinal symptoms. Curr Gastroenterol Rep 4:251–258

Johnson CP, Myers SM (2007) Identification and evaluation of children with autism spectrum disorders. Pediatrics 120:1183–1215. https://doi.org/10.1542/peds.2007-2361

Leekam SR, Nieto C, Libby SJ, Wing L, Gould J (2007) Describing the sensory abnormalities of children and adults with autism. J Autism Dev Disord 37:894–910. https://doi.org/10.1007/s10803-006-0218-7

Li Q, Han Y, Dy ABC, Hagerman RJ (2017) The gut microbiota and autism spectrum disorders. Front Cell Neurosci 11:120. https://doi.org/10.3389/fncel.2017.00120

Luna RA et al (2017) Distinct microbiome-neuroimmune signatures correlate with functional abdominal pain in children with autism spectrum disorder. Cell Mol Gastroenterol Hepatol 3:218–230. https://doi.org/10.1016/j.jcmgh.2016.11.008

Martirosian G et al (2011) Fecal lactoferrin and Clostridium spp. in stools of autistic children. Anaerobe 17:43–45. https://doi.org/10.1016/j.anaerobe.2010.12.003

Mazefsky CA, Schreiber DR, Olino TM, Minshew NJ (2014) The association between emotional and behavioral problems and gastrointestinal symptoms among children with high-functioning autism. Autism 18:493–501. https://doi.org/10.1177/1362361313485164

Nadkarni MA, Martin FE, Jacques NA, Hunter N (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology (Reading, England) 148:257–266. https://doi.org/10.1099/00221287-148-1-257

Parracho HM, Bingham MO, Gibson GR, McCartney AL (2005) Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol 54:987–991. https://doi.org/10.1099/jmm.0.46101-0

Penders J, Vink C, Driessen C, London N, Thijs C, Stobberingh EE (2005) Quantification of Bifidobacterium spp., Escherichia coli and *Clostridium difficile* in faecal samples of breast-fed and formula-fed infants by real-time PCR. FEMS Microbiol Lett 243:141–147. https://doi.org/10.1016/j.femsle.2004.11.052

Rellini E, Tortolani D, Trillo S, Carbone S, Montecchi F (2004) Childhood Autism Rating Scale (CARS) and Autism Behavior Checklist (ABC) correspondence and conflicts with DSM-IV criteria in diagnosis of autism. J Autism Dev Disord 34:703–708

Rosenfeld CS (2015) Microbiome disturbances and autism spectrum disorders. Drug Metab Dispos 43:1557–1571. https://doi.org/10.1124/dmd.115.063826

Sandler RH et al (2000) Short-term benefit from oral vancomycin treatment of regressive-onset autism. J Child Neurol 15:429–435. https://doi.org/10.1177/088307380001500701

Schneider CK, Melmed RD, Barstow LE, Enriquez FJ, Ranger-Moore J, Ostrem JA (2006) Oral human immunoglobulin for children with autism and gastrointestinal dysfunction: a prospective, open-label study. J Autism Dev Disord 36:1053–1064. https://doi.org/10.1007/s10803-006-0141-y

Song Y, Liu C, Finegold SM (2004) Real-time PCR quantitation of clostridia in feces of autistic children. Appl Environ Microbiol 70:6459–6465. https://doi.org/10.1128/aem.70.11.6459-6465.2004

Tomchek SD, Dunn W (2007) Sensory processing in children with and without autism: a comparative study using the short sensory profile. Am J Occup Ther 61:190–200

Van den Abbeele P, Verstraete W, El Aidy S, Geirnaert A, Van de Wiele T (2013) Prebiotics, faecal transplants and microbial network units to stimulate biodiversity of the human gut microbiome. Microb Biotechnol 6:335–340. https://doi.org/10.1111/1751-7915.12049

Wang LW, Tancredi DJ, Thomas DW (2011) The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected

members. J Dev Behav Pediatr 32:351–360. https://doi.org/10.1097/DBP.0b013e31821bd06a

Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA (2013) Increased abundance of Sutterella spp. and Ruminococcus torques in feces of children with autism spectrum disorder. Mol Autism 4:42. https://doi.org/10.1186/2040-2392-4-42

Wasilewska J, Klukowski M (2015) Gastrointestinal symptoms and autism spectrum disorder: links and risks - a possible new overlap syndrome. Pediatr Health Med Ther 6:153–166. https://doi.org/10.2147/phmt.s85717