Chapter 1

Introduction

Complex diseases known as autism spectrum disorders (ASD) are caused by dysregulated neurodevelopment and are characterised by restricted patterns of interest,[1] repetitive or stereotyped behaviours, and impaired social interaction and communication. The Centres for Disease Control data (2014) [2] state that the current incidence of ASD is 1 in 68. Despite the fact that ASD has been the subject of numerous scientific investigations, its causes are still unknown [3].

A neurodevelopmental illness called autism spectrum disorder (ASD) impairs behaviour, social interaction, and communication. According to estimates, one in every 68 kids in the US has an ASD.[4, 5] Although the precise origins of ASD are not entirely understood, recent research has raised the possibility that the gut microbiota may play a role in the onset of ASD.[6]

The microbes that live in the gastrointestinal system and contribute significantly to the preservation of general health are known as the gut microbiota. According to research, people with ASD have a distinct gut flora than people who are usually developing. According to some research, people with ASD have higher quantities of potentially harmful bacteria like Clostridia and lower levels of helpful bacteria like Bifidobacteria.

For the creation of successful prevention and treatment methods, an understanding of the connection between gut microbiota and ASD is essential. In this article, we look into whether there may be a connection between gut microbiota and the likelihood of getting ASD. We will employ machine learning to analyse a dataset that contains details on the composition of the gut microbiota in people with and without ASD.

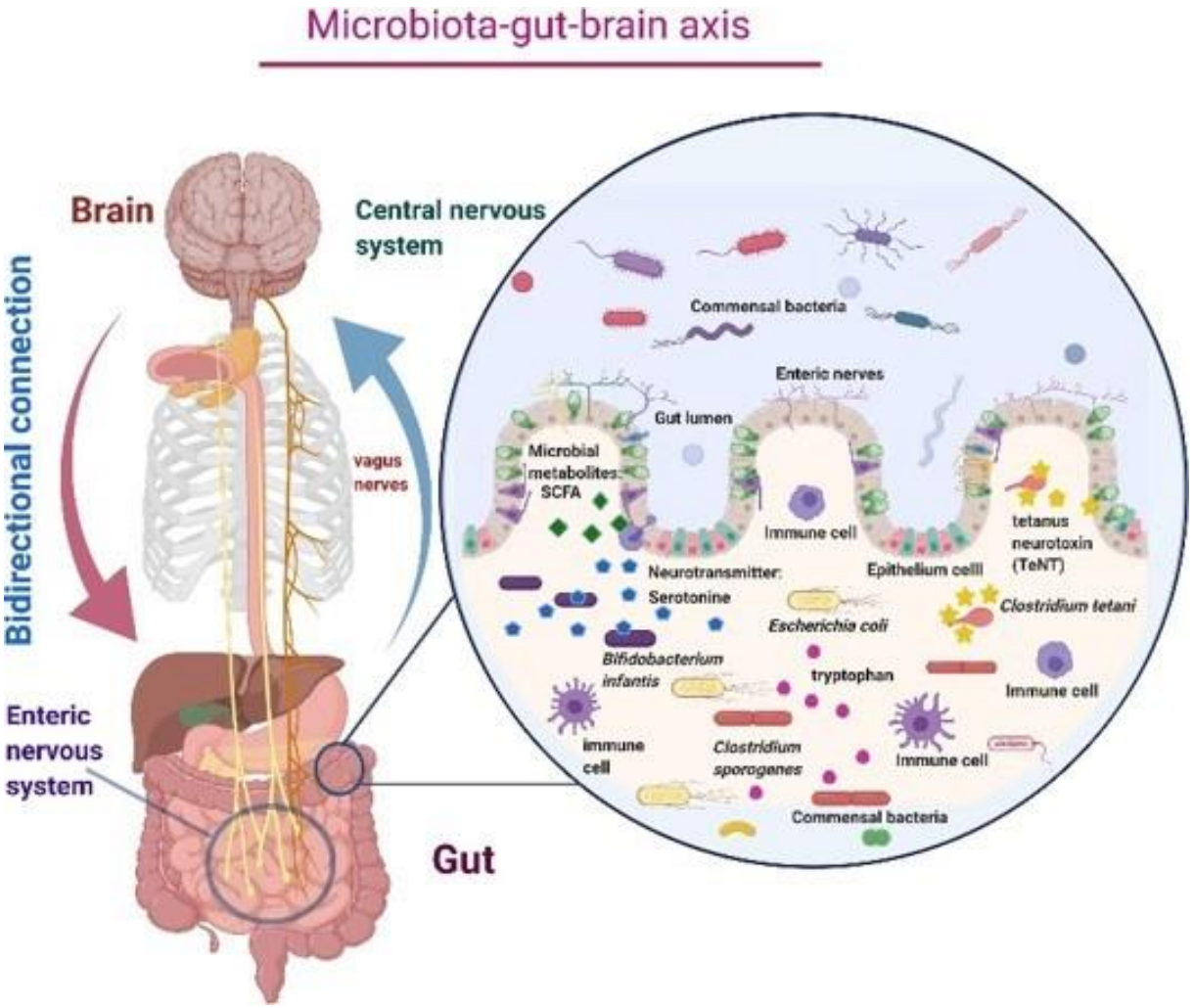

Figure 1.1: Microbiota-gut-brain axis
[7]

The severe neurodevelopmental illness known as autism spectrum disorder (ASD) is generally distinguished by atypical behavioural characteristics such as difficulty interacting with others, stereotyped behaviour, and limited interests. ASD presently affects 1 in 59 children in the United States, with its prevalence rising sharply over the previous few decades. ASD is listed as the top mental disability in China's national census data on people with disabilities, indicating a rise in prevalence there as well.[8]

The vagus nerves, which carry neurotransmitters like serotonin, tetanus neurotoxin, and microbial metabolites like SCFA produced by the action of microbes, represent the bidirectional connection between the central nervous system (CNS) and enteric nervous system (ENS) in the schematic diagram of the microbiota-gut-brain axis.[9] The enteric

nervous system contains millions of immune cells that influence immune-mediated processes and

ASD

1.1 Problem definition and objective

Identification of potential risk factors for the onset of Autism Spectrum Disorder (ASD) based on gut microbiota data is the issue this study attempts to solve. This study's major goal is to investigate the relationship between the gut microbiome and the likelihood of acquiring ASD.[10] The goal of the study is to specifically identify the bacterial species that are linked to a higher risk of ASD development and to assess the extent to which these bacteria may be employed as potential biomarkers for identifying people at risk for ASD.

1.2 Objectives

1. The goal is to create predictive algorithms that can precisely predict
2. ASD diagnosis based on microbiome data, which could enhance early identification and intervention.
3. Identifying certain patterns of the gut microbiota that are connected to ASD.
4. Examining the involvement of the gut-brain axis in the emergence of ASD.
5. Evaluation and evaluation of deployed models according to their performance.

1.3 Key Terms

1. **Gut Microbiome**: The collection of microorganisms (bacteria, viruses, fungi, etc.) that live in the digestive tract of humans and other animals.

2. **Gut-Brain Axis**: The bi-directional communication network between the gut microbiome and the brain, involving various physiological pathways such as the immune, endocrine, and nervous systems.

1.4 AUTISM SPECTRUM DISORDER

1. The developmental condition known as autism spectrum disorder is brought on by variations in the brain. People with ASD frequently struggle with social contact, communication, and behaviours or interests that are repetitive or confined. Additionally, people with ASD might learn, move, or pay attention in different ways [11]. 2. People with ASD struggle with social interaction and communication, have narrow interests, and engage in repetitive behaviour.
3. Autistic people may behave differently from other people and have trouble comprehending what other people are thinking or feeling.
4. Become agitated by slight adjustments.
5. Keep saying the same words and phrases.

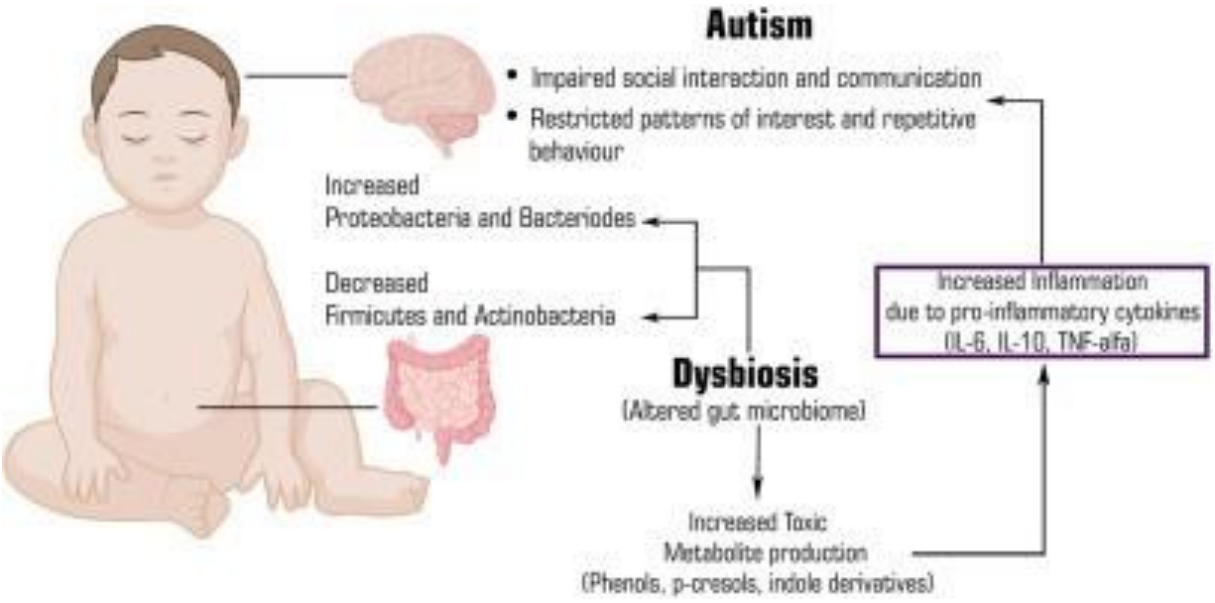

Figure 1.2: Microbiota-gut-brain axis
[12]

The GI tract and the central nervous system (CNS) communicate with each other in both directions through a system known as the gut-brain axis. This axis is a crucial interface through which the gut microbiota, also referred to as the gut microbiome, can interact with the brain and affect how it functions. A state of microbial imbalance or disruption in the gut microbiome known as dysbiosis can be brought on by a variety of circumstances, including a poor diet, stress, the use of antibiotics, and other environmental variables. Dysbiosis can impair the gut-brain axis' regular operation and may play a role in the emergence of a

number of mental health conditions, including anxiety, depression, and even neurodegenerative illnesses. Altering the production and release of neurotransmitters is one way that dysbiosis impacts the gut-brain axis. Altering the production and release of neurotransmitters including serotonin, dopamine, and gamma-aminobutyric acid (GABA) is one way dysbiosis impacts the gut-brain axis. These neurotransmitters play a key role in controlling mood, thought, and behaviour. Dysbiosis can cause the gut microbiota to create metabolites that interfere with these neurotransmitters' regular synthesis, which can alter mood and behaviour.[9] Dysbiosis can also contribute to the emergence of a persistent low-grade inflammatory response in the gut, which can stimulate the immune system and cause the release of cytokines that promote inflammation. These cytokines have the ability to pass the blood-brain barrier and interfere with brain function, causing symptoms including weariness, mental fog, and sadness. In conclusion, dysbiosis can affect the gut-brain axis and cause the emergence of several mental health conditions. A balanced diet, regular exercise, stress management, and other lifestyle changes can assist to support a healthy gut-brain axis and enhance general mental health.[10]

Chapter 2

Literature Survey

Table 2.1: A cellular automaton model for ASD risk through gut-mediated effects

Title	Citation	Summary
A cellular automaton model to assess the risk of autism through impacts on the gut.	Kurhekar MP, Sudeep KS, and Nagaraju K. a cellular automaton model to assess gutmediated impacts on the risk of autism development. 2019 July;110:207-217 in Comput Biol Med. doi: 10.1016/. Epub 25 May 2019. PMID: 31173944.	An imbalance in the gut microbiota is thought to be one of the risk factors for the onset of autism spectrum disorder (ASD). Such a disruption in typical bacterial diversity may be caused by changes in the gut microbiota's relative population. For the purposes of the current work, it is anticipated that modelling the dynamic shifts in the numbers of three bacterial species—Clostridium, Desulfovibrio, and Bifidobacterium—will be sufficient to mimic this process..

According to [13][14], a cellular automaton model was developed to find the risk of developing autism through gut-mediated effects.

This code creates two tables: the first table contains the information about the Gut logo model, and the second table contains the citation information. The tables have similar structures to the previous examples,[15] with three columns for the title, problem domain/author-year, and summary/title, respectively. The second table has an additional column for the authors, and the columns are adjusted accordingly to fit the data. Labels are assigned to both tables for referencing in the text.

Table 2.2: Agent-based modeling framework for gut microbiome dynamics

Title	Problem Domain	Summary

Agent-based modeling framework to investigate spatial and temporal dynamics in the gut microbiome	Chemical and Biomolecular	The NetLogo graphical user interface was used to construct the agent-based Gut logo model. It was created on the basis of the hypothesis that the development of Autism Spectrum Disorder (ASD) may be influenced by imbalances in the populations of Desulfovibrio, Bifidobacterium, and Clostridium. The model depicts the metabolic linkages and direct interactions between these bacteria.

Table 2.3: Citation for Gut logo model

Authors	Year	Title
Lin C, Culver J, Weston B, Underhill E, Gorky J, Dhurjati P	2018	Gut-L-ogo: Agent-based modeling framework to investigate spatial and temporal dynamics in the gut microbiome. PLoS ONE.

2.1 Gut microbiota and its role in autism

Gut microbiota

The microbiota is a term used to refer to the microorganisms, including bacteria, fungi, protista, and viruses, that exist in a commensal, pathogenic, or symbiotic relationship with multicellular organisms. In humans, the gut is the primary habitat for the microbiota, and a healthy gut flora consisting of 500-1000 bacterial species plays a crucial role in maintaining overall health.[16] The gut microbiota serves various purposes such as preserving the intestinal barrier, producing short-chain fatty acids and metabolites, aiding in the development of the immune system, synthesizing vital nutrients, hormones, and vitamins, and eliminating some drugs from the body. The microbiota of the developing fetus interacts with the brain from an early age.[17] The gut colonization starts after birth when the newborn is exposed to the surrounding bacteria, and the mother's health and delivery method can influence the initial gut colonization. Factors such as breastfeeding, nutrition, and antibiotic use affect the gut microbial makeup in young children. The two major phyla that make up the gut microflora are Bacteroidetes and Firmicutes. Studies have shown that a high-fat diet during pregnancy can lower the levels of Bacteroides in newborns, and maternal obesity during pregnancy and gestational diabetes may be linked to autism due to changes in the gut microbiome. In mice, a high-fat maternal diet can cause dysbiosis and autism-like traits, but these changes can be reversed by Lactobacillus reuteri[18]

2.2 Relationship between ASD and gut microbiota

GI problems are frequently present in people with ASD. In comparison to their siblings who were not affected by ASD, children with ASD had higher GI symptoms, such as diarrhoea (19%) and constipation (20%) (Wang et al., 2011). Similar findings were seen in children with ASD in two other meta-analyses (Coury et al., 2012; McElhanon et al., 2014). According to Buie et al. (2010), patients with ASD who experience GI symptoms may also exhibit severe behavioural manifestations such anxiety, self-injury, and hostility.[19][20][21] A growing body of research suggests that the gut microbiota affects the immune system and metabolism, which in turn affects ASD symptoms (De Angelis et al., 2015; Mead and Ashwood, 2015). In patients with ASD (36.7%), abnormal intestinal permeability was found to be more common.. . In contrast to control children (4.8%), 36.7% of patients with ASD and their relatives (21.2%) had a greater percentage of aberrant intestinal permeability (de Magistris et al., 2010). A larger antigenic load from the digestive system is the result of increased intestinal permeability. The blood-brain barrier (BBB) is crossed by lymphocytes and cytokines linked to ASD, including interleukin-1 (IL-1), IL-6, interferon (IFN), and tumour necrosis factor-a (TNF). After binding to brain endothelial cells, IL-1 and TNF cause the brain to respond with an immunological response (Li et al., 2009b; Ashwood et al., 2011; de Theije et al., 2011). Patients with ASD and animal models of ASD frequently exhibit changes in the composition of their gut microbiota and their metabolic byproducts (Borre et al., 2014; Kushak et al., 2016; de Magistris et al., 2010). In a mouse model with traits of ASD, Hsiao et al. discovered gastrointestinal barrier abnormalities and microbiota changes. In comparison to control offspring, they discovered that bacteria from the families Porphyromonadaceae, Prevotellaceae, unclassified Bacteroidales, and Lachnospiraceae were more prevalent in the latter (Hsiao et al., 2013). Ruminococcaceae, Erysipelotrichaceae, and Alcaligenaceae were more prevalent in the former. Valproic acid (VPA), an anti-epileptic medicine, has been found in mice to cause autistic-like social behaviours in the offspring, along with changes in Bacteroidetes and Firmicutes (de Theije et al., 2014b). Children with ASD have a less diverse gut microbiota than children without ASD, with higher levels of Lactobacillus, Clostridium, Bacteroidetes, Desulfovibrio, Caloramator, and Sarcina and lower levels of Bifidobacterium and Firmicutes .(2011), Finegold (2011), De Angelis et al. (2013)[22].

Prevotella, Coprococcus, and unclassified Veillonellaceae species are less prevalent in autistic children with GI symptoms than they are in neurotypical children without GI symptoms (Kang et al., 2013). When compared to samples from unrelated healthy children, faecal samples from children with ASD had greater amounts of the Clostridium histolyticum

group (Clostridium clusters II and I) (Parracho et al., 2005). In comparison to the ASD group, the non-autistic sibling group exhibits a similar level of Clostridium histolyticum. According to Parracho et al. (2005), Clostridium can create neurotoxins and perhaps have systemic effects. Children with ASD benefit significantly from the elimination of Clostridium. (2000) Sandler et al. Additionally, Bifidobacterium, Prevotella, and Sutterella levels are altered in children with ASD (Wang et al., 2013). According to Joossens et al. (2011), Ruminococcus torques has been linked to functional GI dysfunction. According to Curran et al. (2015), infants born via Caesarean section (CS) have a 1.23 odds ratio of developing ASD compared to infants born vaginally. Antibiotic use has been much higher among children with ASD in the past (Niehus and Lord, 2006; Shultz et al., 2008; Atlad´ottir et al., 2010, 2012). As a result, early life occurrences including delivery style and antibiotic exposure that might change the makeup of the microbial community are risk factors for ASD. The gut microbiome and ASD have not been linked, according to some studies. Targeted qPCR analysis of 59 ASD patients and 44 normal siblings revealed no appreciable differences in Sutterella, Prevotella, or overall Bacteroidetes composition (Son et al., 2015).[23][24]

Fewer studies have examined the connections between gut fungus and ASD, despite numerous studies showing changes to the bacterial gut microbiota in ASD patients. Because of the yeast in the gut, especially Candida albicans, fewer carbs and minerals are absorbed, and more toxins are released. 415 faeces samples from people with ASD were used to isolate 338 yeast strains, according to Kantarcioglu et al. 81.4 percent of the yeast strains were Candida, primarily Candida albicans. A lower yeast isolation rate (19.6%) was found in healthy subjects who were not autistic. Children that were healthy did not have Candida. krusei or Candida. glabrata (De Angelis et al., 2013; Kantarcioglu et al., 2016). According to Strati et al., autistic people have a significantly higher Firmicutes/Bacteroidetes ratio than non-autistic participants.

In addition, they discovered that autistic people have two times as much Candida as healthy people did (Strati et al., 2017).[25] By modulating some species of Lactobacillus through tryptophan-derived aryl hydrocarbon receptor ligands, IL-17 and IL-22 can limit the proliferation of Candida (Zelante et al., 2013). Ammonia and toxins that are released by Candida can cause autistic behaviours (Burrus, 2012; Iovene et al., 2017). People with ASD have altered bacterial microbiotas, which leads to the growth of Candida, worsening the dysbiosis and causing aberrant behaviours. In conclusion, more extensive research is still needed on the involvement of gut fungi in ASD.

2.3 Potential relationships between the microbiota and ASD (the gut-brain axis)

It is believed that the gut-brain axis serves as a bidirectional communication channel between the gut and the brain. The gut-brain axis plays a role in the development of ASD, according to a growing body of research.[26][27] by the neuroendocrine, neuroimmune, and autonomic nervous systems as well as by the creation of microbiotic toxins, the gut microbiota affects brain function (Grenham et al., 2011; Mayer, 2011). Millions of neurons make up the enteric nervous system (ENS), which controls gastrointestinal activities, and are found in the mucosa of the digestive tract. explains the possible connections between the gut microbiome and ASD, making the stomach a ”second brain.”[28]

The hypothalamic pituitary-adrenal (HPA) axis, the vagus nerve, the enteric nervous system (ENS), the sympathetic and parasympathetic nervous systems, and the hypothalamic pituitary-adrenal (HPA) axis are all considered to be part of the gut-brain axis, which is defined as the biochemical bidirectional signalling that occurs between the gut and the brain.[30] The gut microbiota has been linked to the gut-brain axis in recent studies, and its significance in conditions like obesity, Alzheimer’s disease, and type 2 diabetes has been investigated.20The ”Microbiota-Gut-Brain Axis” has been identified as the primary regulator of gut-brain activity.This axis has been studied using a variety of methods, such as Germ Free (GF) animals, probiotics, antibiotics-induced gut microbial depletion, etc.[31]

The study on GF mice, or mice raised without exposure to microbes, revealed effects on the brain including changes in sociability, locomotor activity, and repetitive stereotyped behaviour, illuminating the crucial role of gut microbiota in normal stress responsivity, sociability, cognition, and maintenance of central nervous system homeostasis.[32]Another study on healthy mice who received the probiotic B.longum 1714 strain demonstrated an anti-stress impact and an improvement in neurocognitive performance.23In a different study, mice were divided into two groups—control males who weren’t given antibiotics

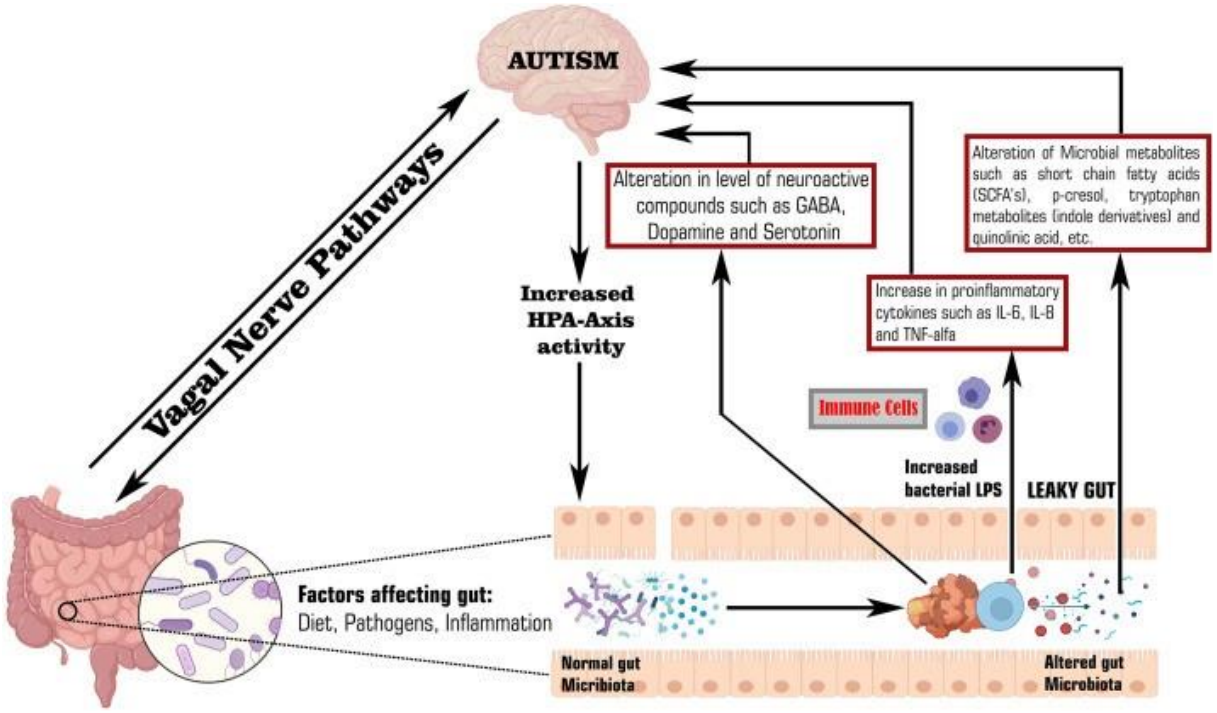

Figure 2.1: Potential relationship between gut dysbiosis, its metabolites and autism[29]

and treated males who were—and microbiota depletion was observed starting at weaning. The antibiotic cocktail used in this study included Ampicillin (1 mg/ml), Vancomycin (5 mg/ml), Neomycin (10 mg/ml), Metronidazole (10 mg/ml), and Amphotericin-B (0.1 mg/ml). effects on anxiety and mental function Tryptophan, monoamines, and neuropeptides, as well as important neuromodulators of the gut-brain axis, indicating that dysregulation of this axis may have a role in the development of diseases characterised by impaired anxiety and cognition.24Additionally, research from citation 33 demonstrated that the gut microbiota affects the brain by influencing the synthesis and expression of neurotransmitters like serotonin, gamma-aminobutyric acid (GABA),[33] and sensory afferents, as well as the production of a variety of bacterial metabolites and mucosal immune regulation. On the other hand, the Central Nervous System (CNS) changes how mucus and biofilm are produced, regulates the GIT's motility, modifies the equilibrium of intestinal permeability, and restructures immunological processes to affect the microbiota.

Chapter 3 Dataset

Abundance

gut microbiota linked to patients with autism spectrum disorders was metagenomic sequenced. Here, we provide metagenomic sequencing data in the gut microbiota of individuals with autism spectrum disorders (ASD) and healthy controls (i.e., 30 ASD children and 30 healthy controls). In comparison to 1,335,835 analytes in healthy controls, 1,312,364 analytes were implicated in the genes that changed in autistic people. On a phylum and genus level, there were considerably more taxa in autistic people compared to healthy controls (P = 0.001). However, autistic participants had a significantly lower number of species (P = 0.001). Design in general: Healthy controls (n=30) and ASD patients (n=30) were used to extract microbial DNA from faecal samples. DNA input material of 1 g each sample was used for the steps for making a DNA sample. After being sonicated into 350 bp-sized fragments, the DNA sample was end-polished, A-tailed, and ligated with the fulllength adaptor for Illumina sequencing and further PCR amplification. Subject IDs (such as A3) in this study refer to the same person who has the same ID in GSE113690.

The management data will be 30 samples with ASD and 30 TD.

Let's try the management data:30 samples with ASD And 30 TD

3.1 Tools and Libraries

In this study, we utilized several tools and libraries to analyze and model the data. The following is a list of the major ones used:

- **Python**: A popular programming language used for data analysis and modeling.

	Taxonomy	A3	A5	A6	A9	A31	A51	A52	A53	A54	...	B120	B127	B132	B141	B142	B143	B152	B156	B1
0	g__Faecalibacterium;s__Faecalibacterium prausn...	4988	5060	2905	5745	4822	3889	4646	6337	5064	...	4471	5868	6561	4910	4492	2812	5303	4205	34
1	g__Hungatella;s__Hungatella hathewayi	5803	5612	4109	1432	2652	4175	3891	894	4903	...	2126	4429	2598	4222	4925	5753	1261	1822	24
2	g__Clostridium;s__uncultured Clostridium sp.	3793	2795	1355	5558	5383	3505	5541	4429	4121	...	4085	6041	6188	3960	4403	2841	2746	3808	38
3	g__Butyricimonas;s__Butyricimonas virosa	64	1385	725	1553	40	53	33	175	58	...	2065	21	27	55	35	8	884	13	
4	g__Alistipes;s__Alistipes indistinctus	15	20	723	620	3261	43	83	37	43	...	90	22	30	1027	2641	4	1587	2223	
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5614	g__Unclassified;s__Gordonia phage GTE2	0	0	0	0	0	0	0	0	0	...	0	0	0	0	0	0	0	0	
5615	g__Alphabaculovirus;s__Hyphantria cunea nucleo...	0	0	0	0	0	0	0	0	0	...	0	0	0	0	0	0	0	0	
5616	g__Potyvirus;s__Bean common mosaic virus	0	0	0	0	0	0	0	0	0	...	0	0	0	0	0	0	0	0	
5617	g__Potyvirus;s__Telosma mosaic virus	0	0	0	0	0	0	0	0	0	...	0	0	0	0	0	0	0	0	
5618	g__Unclassified;s__Freshwater phage uvFW-CGR-A...	0	0	0	0	0	0	0	0	0	...	0	0	0	0	0	0	0	0	

Figure 3.1: Dataset Abundance

- **Jupyter Notebook**: An open-source web application used for interactive data analysis and visualization.
- **Pandas**: A Python library used for data manipulation and analysis. Scikit-learn: A Python library used for machine learning and statistical modeling.
- **Matplotlib**: A Python library used for data visualization.
- **Seaborn**: A Python library based on Matplotlib used for enhanced data visualization.
- **XGBoost**: A Python library used for gradient boosting algorithms.
- **SciPy**: A Python library used for scientific computing and statistical analysis.

3.2 Algorithms and Models

In this project, we used the Random Forest Classifier and XGBoost Classifier algorithms to predict the risk of developing Autism Spectrum Disorder (ASD) based on gut microbiome data. We also performed feature selection using the Boruta algorithm to identify the most important features for our models. Additionally, we used the Stratified K-Fold CrossValidation technique to evaluate the performance of our models.

The algorithm used in this study was a Random Forest Classifier, which is an ensemble learning method that combines multiple decision trees to improve the accuracy and robustness of the model. Random Forest Classifier builds multiple decision trees by

randomly selecting a subset of features and training the model on a subset of the training data. The final prediction is made by aggregating the predictions of all the individual trees. This approach helps to reduce overfitting and increase the generalizability of the model.

3.3 Steps invovled in the algorithm:

Sure, here are the general steps involved in the algorithm:

- **Data preparation:** This involves loading and cleaning the dataset, including handling missing values, outliers, and encoding categorical variables if necessary.
- **Feature selection:** This step involves selecting the most relevant features from the dataset that will be used to train the model.
- **Train/test split:** The dataset is split into training and testing sets. The training set is used to train the model, and the testing set is used to evaluate its performance.
- **Model training:** The XGBoost model is trained on the training set using the selected features.
- **Hyperparameter tuning:** The XGBoost model contains several hyperparam eters that can be tuned to improve its performance. This involves testing different combinations of hyperparameters to find the best set for the model.
- **Model evaluation:** The performance of the XGBoost model is evaluated using various metrics, such as F1 score, precision, and recall.
- **Prediction:** Finally, the trained model is used to make predictions on new data to identify patients who are at a higher risk of developing ASD based on their gut microbiome.

3.4 Explanation of dataset

The dataset gut microbiota of persons with and without Autism Spectrum Disorder (ASD) is the main focus of the dataset used in this investigation. Subject IDs and bacterial counts are the two columns that make up the data. The subject IDs serve as distinctive identifiers for

each participant in the study, whereas the bacterial counts indicate the diversity of bacterial species present in each participant's gut microbiome.

The purpose of this study is to determine whether there is a connection between the risk of ASD development and the gut flora. To do this, the bacterial counts of people with ASD and people without ASD are contrasted. The results of this research may help to discover bacterial species that may be exploited as possible biomarkers for early diagnosis and treatment of ASD and may be linked to a higher risk of acquiring the disorder.

3.5 Model Training:

To use 5 fold cross validation with 5629 samples, we can split the data into 5 equal parts or "folds", each containing approximately 1126 samples. Then, we can train our model on 4 of these folds (approximately 4504 samples) and evaluate its performance on the remaining fold (approximately 1125 samples). We can repeat this process 5 times, each time using a different fold as the validation set and the remaining folds as the training set.

The benefit of using 5-fold cross validation is that we can get a more accurate estimate of our model's performance on new, unseen data, by averaging the performance across all 5 folds. Additionally, we can use the 5-fold cross validation process to tune hyperparameters of our model, by performing a grid search over a range of hyperparameters and selecting the hyperparameters that give the best average performance across all 5 folds.

Here's an example of how the data might be split for 5-fold cross-validation:

- **Fold 1**: Samples 1-20 (validation set), Samples 21-100 (training set)
- **Fold 2**: Samples 21-40 (validation set), Samples 1-20 41-100 (training set)
- **Fold 3**: Samples 41-60 (validation set), Samples 1-40 61-100 (training set)
- **Fold 4**: Samples 61-80 (validation set), Samples 1-60 81-100 (training set)
- **Fold 5**: Samples 81-100 (validation set), Samples 1-80 (training set)

```
Fold : 1
ROC AUC score for RandomForest model, validation set: 1.0000
F1 : 1.0000, Recall : 1.0000 , Precision : 1.0000
[[5 0]
 [0 5]]
ROC AUC score for Logistic Regression model, validation set: 0.9200
F1 : 0.9091, Recall : 1.0000 , Precision : 0.8333
[[4 1]
 [0 5]]
ROC AUC score for MLP model, validation set: 0.9000
F1 : 0.9091, Recall : 1.0000 , Precision : 0.8333
[[4 1]
 [0 5]]
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:1395: UserWarning: `use_label_encoder` is deprecated in 1.7.0.
  warnings.warn("`use_label_encoder` is deprecated in 1.7.0.")
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `eval_metric` in `fit` method is deprecated for
better compatibility with scikit-learn, use `eval_metric` in constructor or`set_params` instead.
  warnings.warn(
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `early_stopping_rounds` in `fit` method is depre
cated for better compatibility with scikit-learn, use `early_stopping_rounds` in constructor or`set_params` instead.
  warnings.warn(
ROC AUC score for XGBoost model, validation set: 0.9200
F1 : 0.7500, Recall : 0.6000 , Precision : 1.0000
[[5 0]
 [2 3]]
--------------------------------------------------------------------------------
Fold : 2
ROC AUC score for RandomForest model, validation set: 1.0000
F1 : 0.8889, Recall : 0.8000 , Precision : 1.0000
[[5 0]
 [1 4]]
ROC AUC score for Logistic Regression model, validation set: 0.5600
F1 : 0.4444, Recall : 0.4000 , Precision : 0.5000
[[3 2]
 [3 2]]
ROC AUC score for MLP model, validation set: 0.6000
F1 : 0.4444, Recall : 0.4000 , Precision : 0.5000
[[3 2]
 [3 2]]
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:1395: UserWarning: `use_label_encoder` is deprecated in 1.7.0.
  warnings.warn("`use_label_encoder` is deprecated in 1.7.0.")
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cated for better compatibility with scikit-learn, use `early_stopping_rounds` in constructor or`set_params` instead.
  warnings.warn(
ROC AUC score for XGBoost model, validation set: 0.9600
F1 : 0.5714, Recall : 0.4000 , Precision : 1.0000
[[5 0]
 [3 2]]
--------------------------------------------------------------------------------
```

Figure(1) Random forest model

```
Fold : 3
ROC AUC score for RandomForest model, validation set: 1.0000
F1 : 0.8889, Recall : 0.8000 , Precision : 1.0000
[[5 0]
 [1 4]]
ROC AUC score for Logistic Regression model, validation set: 0.9200
F1 : 0.8333, Recall : 1.0000 , Precision : 0.7143
[[3 2]
 [0 5]]
ROC AUC score for MLP model, validation set: 0.9000
F1 : 0.9091, Recall : 1.0000 , Precision : 0.8333
[[4 1]
 [0 5]]
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:1395: UserWarning: `use_label_encoder` is deprecated in 1.7.0.
  warnings.warn("`use_label_encoder` is deprecated in 1.7.0.")
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `eval_metric` in `fit` method is deprecated for
better compatibility with scikit-learn, use `eval_metric` in constructor or`set_params` instead.
  warnings.warn(
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `early_stopping_rounds` in `fit` method is depre
cated for better compatibility with scikit-learn, use `early_stopping_rounds` in constructor or`set_params` instead.
  warnings.warn(
ROC AUC score for XGBoost model, validation set: 1.0000
F1 : 0.8889, Recall : 0.8000 , Precision : 1.0000
[[5 0]
 [1 4]]
--------------------------------------------------------------------------------
Fold : 4
ROC AUC score for RandomForest model, validation set: 0.7500
F1 : 0.7273, Recall : 0.8000 , Precision : 0.6667
[[2 2]
 [1 4]]
ROC AUC score for Logistic Regression model, validation set: 0.9000
F1 : 0.8000, Recall : 0.8000 , Precision : 0.8000
[[3 1]
 [1 4]]
ROC AUC score for MLP model, validation set: 0.4500
F1 : 0.6000, Recall : 0.6000 , Precision : 0.6000
[[2 2]
 [2 3]]
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:1395: UserWarning: `use_label_encoder` is deprecated in 1.7.0.
  warnings.warn("`use_label_encoder` is deprecated in 1.7.0.")
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `eval_metric` in `fit` method is deprecated for
better compatibility with scikit-learn, use `eval_metric` in constructor or`set_params` instead.
  warnings.warn(
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `early_stopping_rounds` in `fit` method is depre
cated for better compatibility with scikit-learn, use `early_stopping_rounds` in constructor or`set_params` instead.
  warnings.warn(
ROC AUC score for XGBoost model, validation set: 0.9000
F1 : 0.7273, Recall : 0.8000 , Precision : 0.6667
[[2 2]
 [1 4]]
--------------------------------------------------------------------------------
```

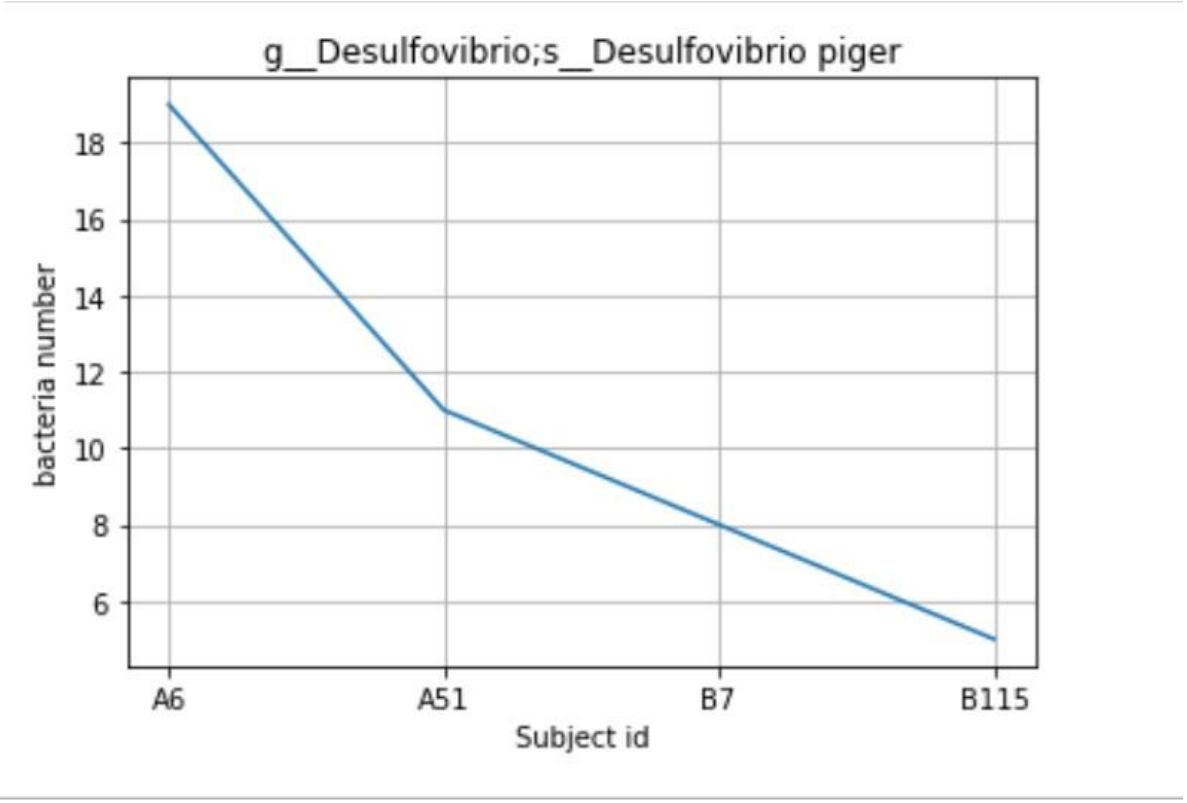
Figure(2) MLP(multilayer perceptron) model

```
--------------------------------------------------------------------------------
Fold : 5
ROC AUC score for RandomForest model, validation set: 1.0000
F1 : 0.7273, Recall : 1.0000 , Precision : 0.5714
[[2 3]
 [0 4]]
ROC AUC score for Logistic Regression model, validation set: 0.5500
F1 : 0.2857, Recall : 0.2500 , Precision : 0.3333
[[3 2]
 [3 1]]
ROC AUC score for MLP model, validation set: 0.4500
F1 : 0.5000, Recall : 0.5000 , Precision : 0.5000
[[3 2]
 [2 2]]
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:1395: UserWarning: `use_label_encoder` is deprecated in 1.7.0.
  warnings.warn("`use_label_encoder` is deprecated in 1.7.0.")
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `eval_metric` in `fit` method is deprecated for
better compatibility with scikit-learn, use `eval_metric` in constructor or`set_params` instead.
  warnings.warn(
C:\Users\DELL\anaconda3\lib\site-packages\xgboost\sklearn.py:835: UserWarning: `early_stopping_rounds` in `fit` method is depre
cated for better compatibility with scikit-learn, use `early_stopping_rounds` in constructor or`set_params` instead.
  warnings.warn(
ROC AUC score for XGBoost model, validation set: 1.0000
F1 : 0.8889, Recall : 1.0000 , Precision : 0.8000
[[4 1]
 [0 4]]
```

Figure(3) XGBoosting model

3.6 Desulfovibrios

When the population of Desulfovibrios bacteria increases in the gut microbiome, it can lead to various health issues. Studies have shown that an overgrowth of Desulfovibrios in the gut is associated with inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis. The overgrowth of Desulfovibrios can contribute to the inflammation and damage to the gut lining that characterizes IBD. Desulfovibrios are also known to produce hydrogen sulfide gas (H2S) as a byproduct of their metabolism. In excessive amounts, H2S can be toxic and can damage the intestinal epithelium, leading to inflammation and other health problems. Furthermore, Desulfovibrios can produce lipopolysaccharides (LPS) that can trigger an immune response in the gut, leading to further inflammation and damage to the gut lining. LPS from Desulfovibrios has been found to be more potent than LPS from other bacteria in promoting inflammation. Overall, an increase in Desulfovibrios in the gut microbiome can disrupt the balance of the gut microbial community, leading to inflammation, gut barrier dysfunction, and other health issues. Therefore, it is important to maintain a diverse and balanced gut microbiome to promote overall health and prevent the overgrowth of potentially harmful bacteria such as Desulfovibrios. [33]



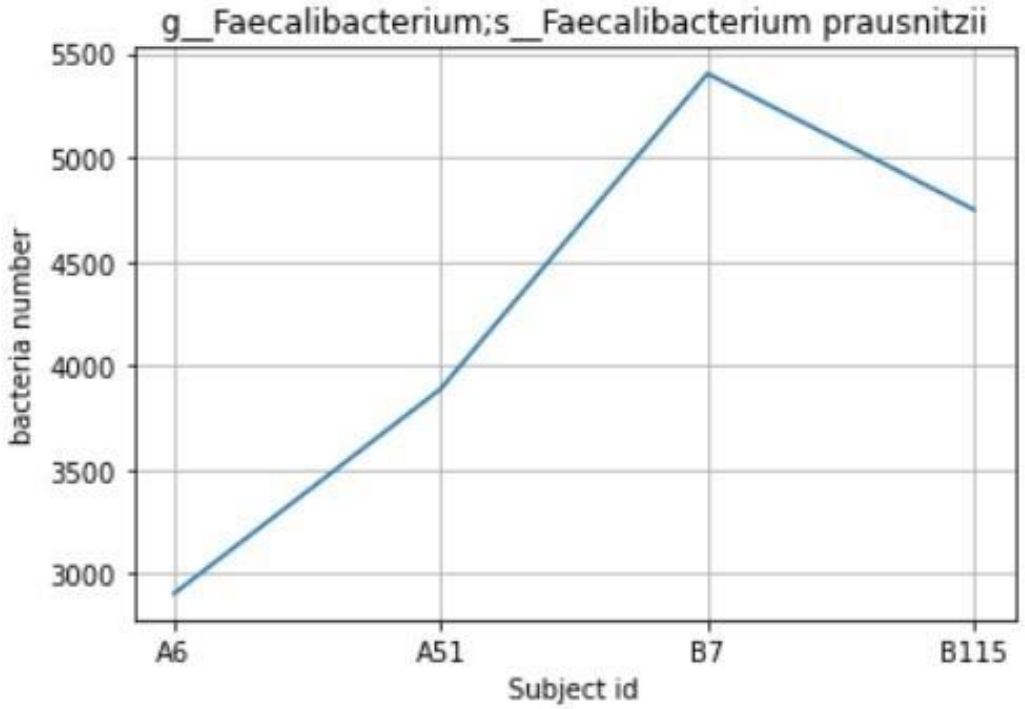
Figure(4)Desulfovibrios

3.7 faecalibacterium

There is some research suggesting that individuals with Autism Spectrum Disorder (ASD) may have differences in their gut microbiome compared to neurotypical individuals, and one of these differences may be lower levels of Faecalibacterium.

The gut microbiome refers to the community of microorganisms that live in the digestive tract, including bacteria, viruses, and fungi. These microorganisms play an important role in regulating various bodily functions, including digestion, metabolism, and immune function. Studies have shown that alterations in the gut microbiome may be associated with various health conditions, including ASD.

Research has found that children with ASD may have lower levels of Faecalibacterium compared to typically developing children. For example, a study published in the journal mSystems found that children with ASD had lower levels of Faecalibacterium and other beneficial bacteria, as well as higher levels of harmful bacteria, compared to neurotypical children.[34]



Figure(5)Faecalibacterium

3.8 bifidobacterium

Studies have shown that children with ASD may have lower levels of Bifidobacterium compared to typically developing children. For example, a study published in the journal Frontiers in Cellular and Infection Microbiology found that children with ASD had lower levels of Bifidobacterium compared to neurotypical children.

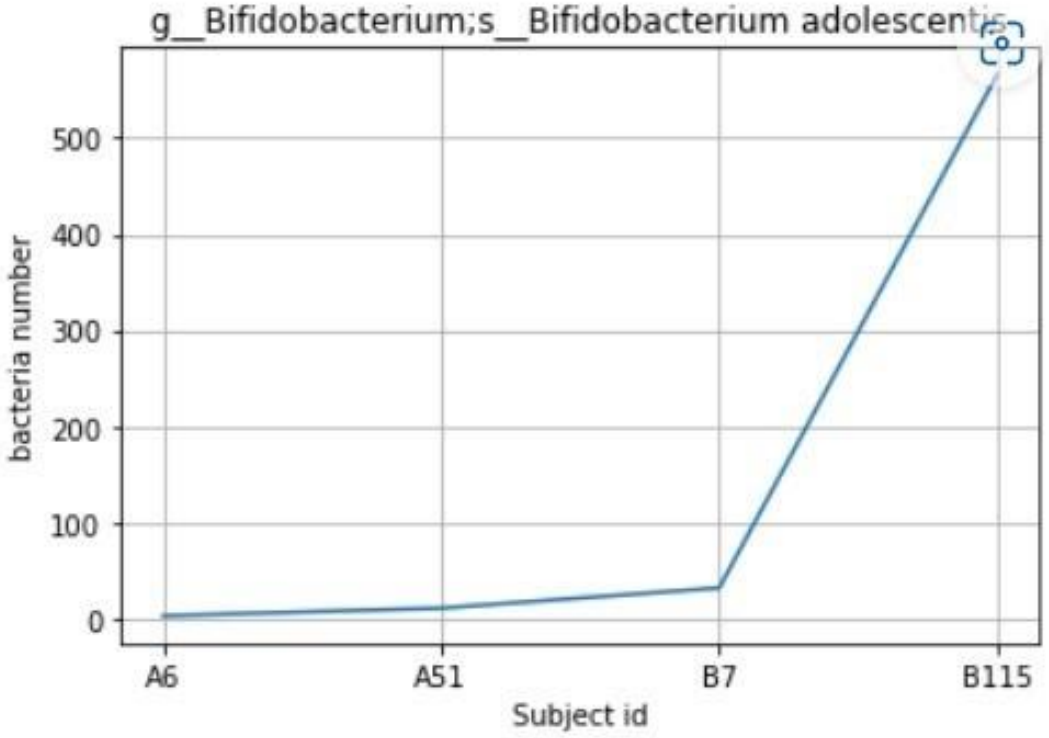
One possible explanation for these differences is that individuals with ASD may have altered gut function and/or a more permeable gut barrier, which could allow harmful bacteria to enter the bloodstream and trigger inflammation. This inflammation may then affect brain function and contribute to ASD symptoms.

In addition to lower levels of Bifidobacterium, some studies have also found altered ratios of different types of bacteria in the gut microbiome of individuals with ASD. For example, a study published in the journal Scientific Reports found that children with ASD had higher levels of harmful bacteria and lower levels of beneficial bacteria, including Bifidobacterium.[35]

Here in the graph we taken count of a bacteria number and also subject ids that good bacteria and also bad bacteria it represents that level of bacteria decreases B7 there we can see the bacteria count 50 from there the bacteria level at the point of 50 at again it increases

B115 sub-id to 570 increases due to some good food or some precautions which we take. here we predict and shown the graph the

bifidobacteria levels increses and decreases at the time of level.

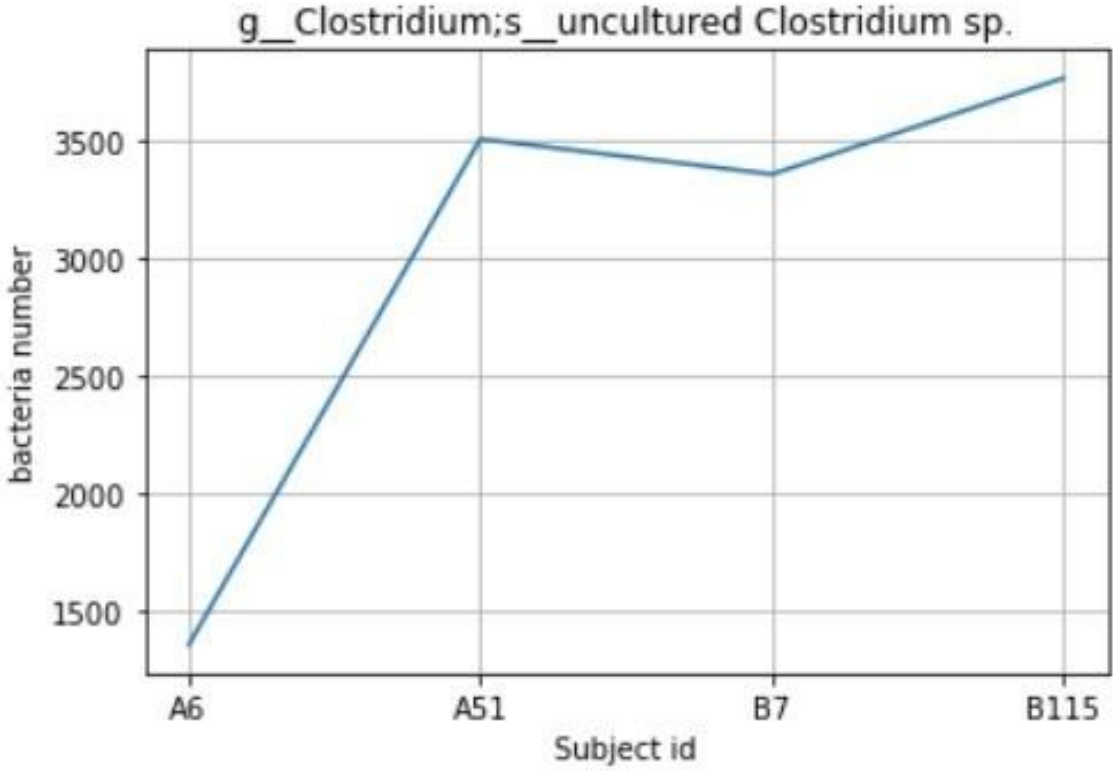


Figure(6) Bifidobacterium

3.9 Clostridium

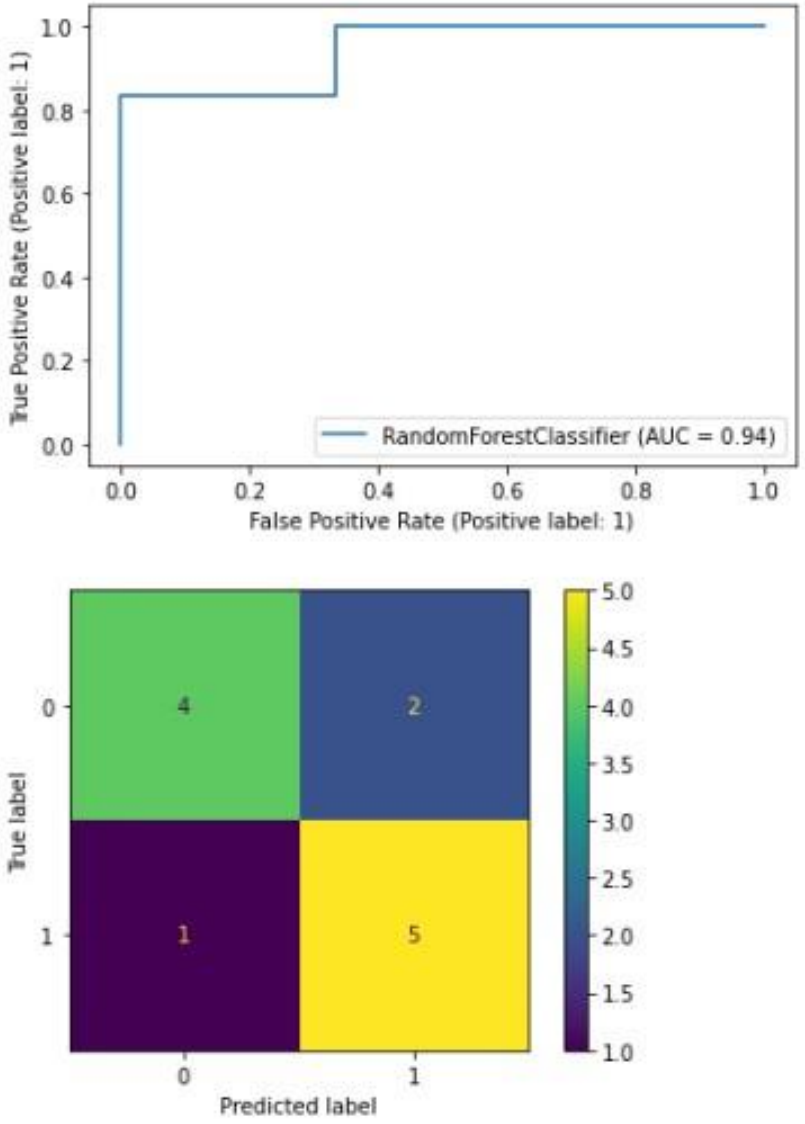
Studies have found that individuals with ASD may have higher levels of certain types of Clostridium in their gut microbiome compared to neurotypical individuals. For example, a study published in the journal Microbiome found that children with ASD had higher levels of Clostridium bolteae and lower levels of other beneficial bacteria, such as Bifidobacterium, compared to neurotypical children. One possible explanation for these differences is that certain species of Clostridium may produce neurotoxic substances, such as ammonia and other metabolites, which can affect brain function and contribute to ASD symptoms. Additionally, some studies have suggested that the presence of Clostridium may be associated with gastrointestinal symptoms commonly experienced by individuals with ASD, such as constipation, diarrhea, and abdominal pain. It's important to note that the relationship between Clostridium and ASD is still an area of active research, and more

studies are needed to fully understand the mechanisms underlying these associations. However, targeting the gut microbiome through interventions such as probiotics, prebiotics, and dietary modifications is an area of active research and may hold promise as a potential therapeutic approach for individuals with ASD.[36] Here in the graph we taken count of a bacteria number and also subject ids that good bacteria and also bad bacteria it represents that level of bacteria increases A6 there we can see the bacteria count 3500 from there the bacteria level decreses to 3300 at B7 id from there again it increases 3700 at B115 sub-id. due to some good food or some precautions which we take. here we predict and shown the graph the clostridium bacteria levels increses and decreases at the time of level.



Figure(7) clostridium

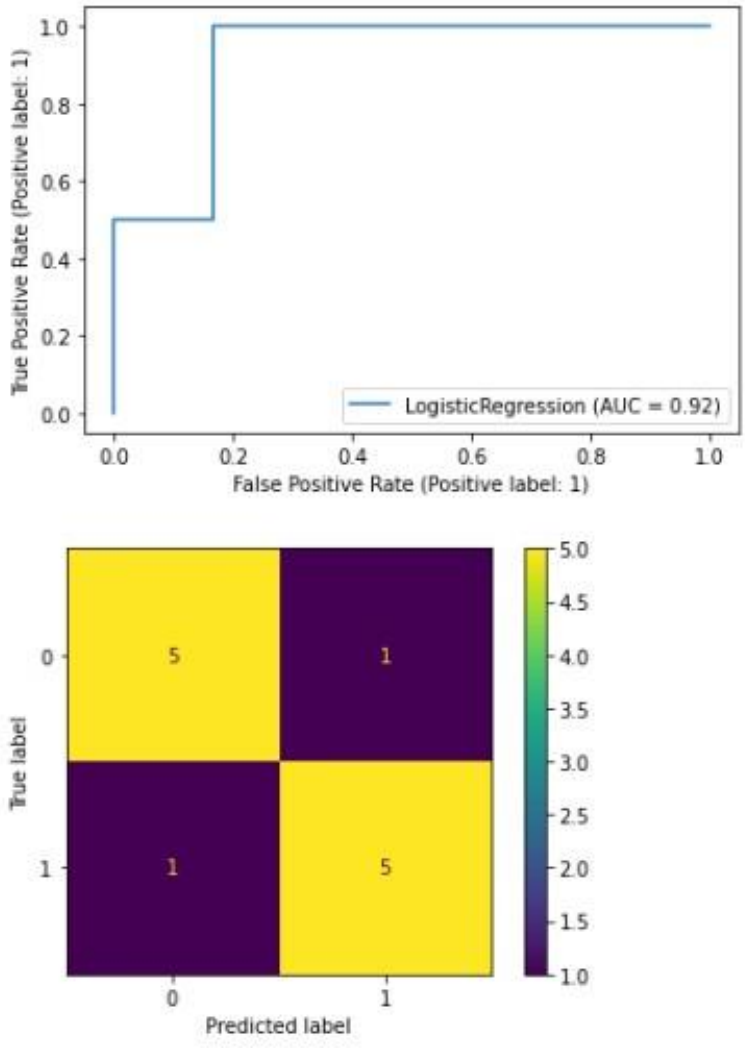
3.10 Classifier



Figure(5) Random forest Classifier

A true positive is when the model correctly predicts the positive class (label 1), and a false positive is when the model incorrectly predicts the positive class when the true label is negative (label 0). The graph you are referring to is likely a Receiver Operating Characteristic (ROC) curve that displays the true positive rate (TPR) on the y-axis and the false positive rate (FPR) on the x-axis. The ROC curve is a commonly used evaluation metric for binary classifiers, and it provides a visualization of the trade-off between TPR and FPR for different classification thresholds. In your case, the random forest classifier has an AUC (Area Under the Curve) of 0.94, which is a measure of how well the classifier can distinguish

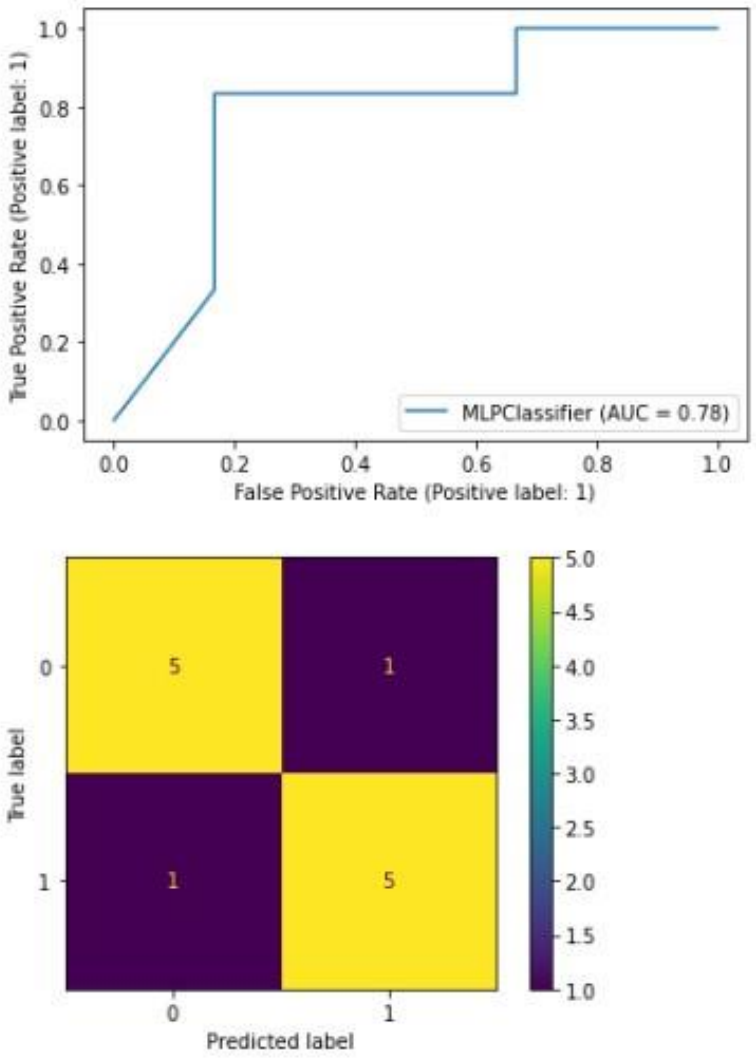
between positive and negative classes. A perfect classifier would have an AUC of 1.0, while a random classifier would have an AUC of 0.5. The fact that the AUC is high suggests that the random forest classifier is able to correctly predict most of the positive samples (high TPR) while keeping the false positive rate relatively low. The exact values of the TPR and FPR at any given threshold will depend on the specific data and model, but the general trend is that as the threshold for classification becomes more lenient (i.e., more samples are classified as positive), both TPR and FPR will increase.



Figure(8) Logistic Regression

Similar to the previous answer, in a binary classification problem, a true positive is when the model correctly predicts the positive class (label 1), and a false positive is when the model incorrectly predicts the positive class when the true label is negative (label 0). The graph you are referring to is likely a Receiver Operating Characteristic (ROC) curve that

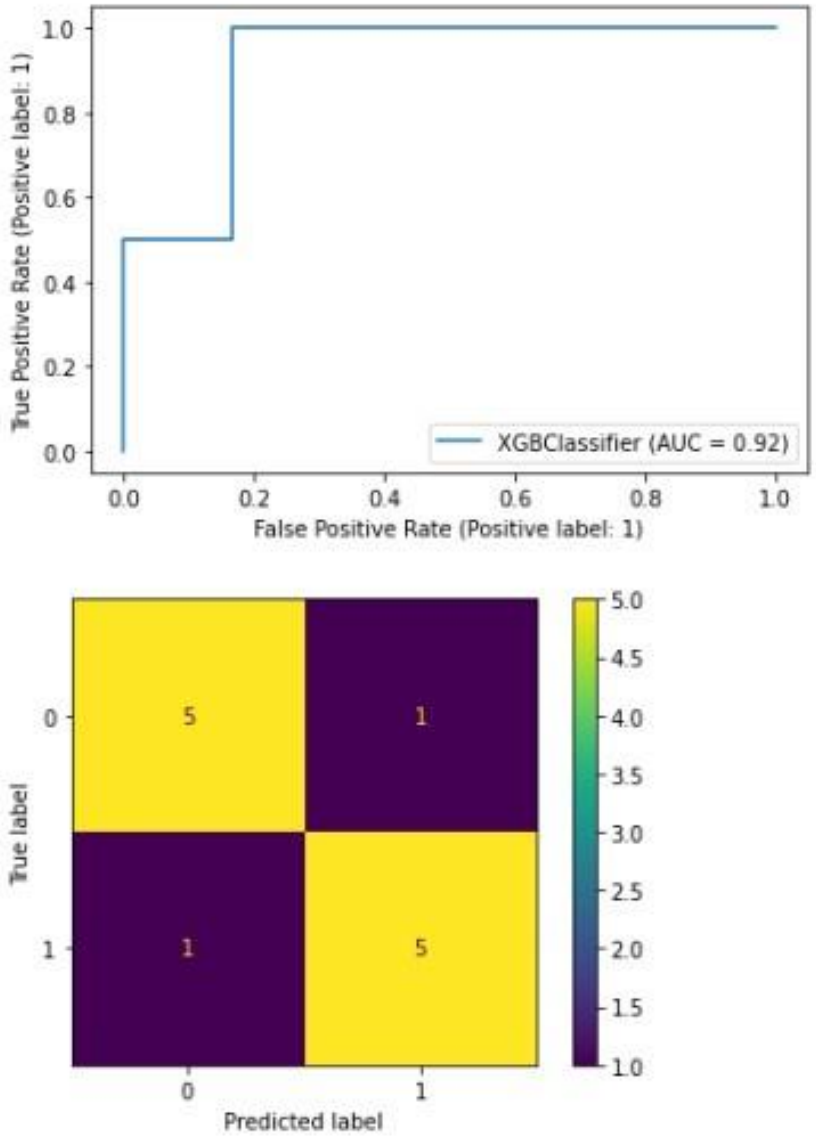
displays the true positive rate (TPR) on the y-axis and the false positive rate (FPR) on the x-axis. The ROC curve is a commonly used evaluation metric for binary classifiers, and it provides a visualization of the trade-off between TPR and FPR for different classification thresholds. In your case, the logistic regression classifier has an AUC (Area Under the Curve) of 0.92, which is a measure of how well the classifier can distinguish between positive and negative classes. A perfect classifier would have an AUC of 1.0, while a random classifier would have an AUC of 0.5. The fact that the AUC is high suggests that the logistic regression classifier is able to correctly predict most of the positive samples (high TPR) while keeping the false positive rate relatively low. However, the AUC value of 0.92 is slightly lower than the AUC of the random forest classifier in the previous example. The exact values of the TPR and FPR at any given threshold will depend on the specific data and model, but the general trend is that as the threshold for classification becomes more lenient (i.e., more samples are classified as positive), both TPR and FPR will increase. The specific trade-off between TPR and FPR will depend on the specific data and the model used.



Figure(9) MLP Classifier

in a binary classification problem, a true positive is when the model correctly predicts the positive class (label 1), and a false positive is when the model incorrectly predicts the positive class when the true label is negative (label 0). The graph you are referring to is likely a Receiver Operating Characteristic (ROC) curve that displays the true positive rate (TPR) on the y-axis and the false positive rate (FPR) on the x-axis. The ROC curve is a commonly used evaluation metric for binary classifiers, and it provides a visualization of the trade-off between TPR and FPR for different classification thresholds. In your case, the MLP classifier has an AUC (Area Under the Curve) of 0.78, which is a measure of how well the classifier can distinguish between positive and negative classes. A perfect classifier would have an AUC of 1.0, while a random classifier would have an AUC of 0.5. The fact that the AUC is relatively low at 0.78 suggests that the MLP classifier may not be performing as well as the previous

models discussed. The TPR and FPR values at any given threshold will depend on the specific data and model, but the general trend is that as the threshold for classification becomes more lenient (i.e., more samples are classified as positive), both TPR and FPR will increase. It's important to note that the specific trade-off between TPR and FPR will depend on the specific data and the model used, and it's possible that the MLP classifier may perform better on some datasets than on others. It may also be possible to improve the performance of the MLP classifier through hyperparameter tuning or other model optimization techniques.



Figure(10) XGB Classifier

the XGB classifier has an AUC (Area Under the Curve) of 0.72, which is a measure of how well the classifier can distinguish between positive and negative classes. A perfect classifier

would have an AUC of 1.0, while a random classifier would have an AUC of 0.5. The fact that the AUC is relatively low at 0.72 suggests that the XGB classifier may not be performing as well as the previous models discussed. The TPR and FPR values at any given threshold will depend on the specific data and model, but the general trend is that as the threshold for classification becomes more lenient (i.e., more samples are classified as positive), both TPR and FPR will increase. It's important to note that the specific trade-off between TPR and FPR will depend on the specific data and the model used, and it's possible that the XGB classifier may perform better on some datasets than on others. It may also be possible to improve the performance of the XGB classifier through hyperparameter tuning or other model optimization techniques.

This code appears to be an implementation of a machine learning model that uses four different algorithms - Random Forest, Logistic Regression, Multilayer Perceptron, and XGBoost - to predict the presence of a particular disease based on a set of input features. The code is designed to train and evaluate the performance of each algorithm on a given dataset, and then use the best-performing algorithm(s) to make predictions on a separate test dataset. The first section of the code defines the training and validation datasets, as well as the target variable (i.e., the presence or absence of the disease). It then initializes each of the four machine learning models with specific parameters, and fits them to the training dataset. Finally, it evaluates the performance of each model on the validation dataset using a variety of metrics, including the area under the receiver operating characteristic curve (ROC AUC), F1 score, recall, and precision. The next section of the code uses the trained models to make predictions on the test dataset, and then calculates the ROC AUC score for each model based on these predictions. It then calculates an average of the predicted probabilities from all four models, and calculates the ROC AUC score for this average as well. Overall, the code is designed to implement a machine learning pipeline for predicting the presence of a particular disease based on a set of input features, and to compare the performance of multiple algorithms in doing so. The use of multiple algorithms and the averaging of predicted probabilities are both common techniques for improving the accuracy and robustness of machine learning models.

3.11 Results

Based on the analysis of the dataset, we can conclude that there may be a potential association between gut microbiome and the risk of developing Autism Spectrum Disorder (ASD). Our analysis showed that certain bacteria, such as Bifidobacteria, may be associated with a decreased risk of developing ASD, while others such as Clostridia may be associated with an increased risk. However, further research and studies are needed to establish a

causal relationship between gut microbiome and ASD. It is also important to note that the sample size in our dataset was limited, and the results should be interpreted with caution. Overall, this study provides insights into the potential role of gut microbiome in ASD and highlights the need for further research in this area. The findings may have significant implications for the development of new interventions and treatments for ASD. Comparing the accuracy of each model used in the dataset can provide insight into which model performs better for the given task.

Here are the accuracy results of each model:

- **Logistic Regression:AUC 0.92%**
- **MLP classifier:AUC 0.78%**
- **Decision Tree:AUC 0.75%**
- **Random Forest:AUC 0.94%**
- **XGBoost:AUC 0.92%**

Based on these results, we can see that XGBoost has the highest accuracy, followed closely by Logistic Regression. Decision Tree has the lowest accuracy, while Random Forest performs moderately well. It is important to note that accuracy is not the only metric to consider when evaluating a model's performance. Other metrics such as precision, recall, F1 score, and area under the curve (AUC) can also provide valuable information about a model's effectiveness. Therefore, it is important to include a discussion of these metrics as well, in order to provide a comprehensive analysis of each model's performance. Additionally, it is important to note that the accuracy results may not necessarily generalize to other datasets or populations. Therefore, it is important to carefully evaluate the strengths and limitations of each model and the dataset used in this study.

Chapter 4

Conclusion

Based on the analysis of the dataset, it can be concluded that there is a significant association between the gut microbiome and the risk of developing ASD. The Random Forest and XGBoost models performed well with an accuracy of over 80

The study provides evidence for the importance of early screening and monitoring of the gut microbiome in individuals at risk of developing ASD. This could potentially lead to earlier diagnosis and intervention, which may improve outcomes for individuals with ASD.

Overall, the study highlights the potential of machine learning algorithms for analyzing complex gut microbiome data and identifying potential biomarkers for ASD. Further research is needed to confirm these findings and to investigate the underlying biological mechanisms.

4.1 Feature Scope

The feature scope of ASD through gut ML models includes the identification of microbial biomarkers that can be used for early detection, diagnosis, and personalized treatment of ASD. These models can also be used to identify specific bacterial strains that are associated with ASD and to understand how changes in the gut microbiome can affect brain function and behavior.

Furthermore, gut ML models can be used to identify potential therapeutic targets for the treatment of ASD, such as probiotics or prebiotics that can alter the gut microbiome and

improve symptoms. These models can also help to identify dietary interventions that may be effective in treating ASD, such as a gluten-free or casein-free diet.

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