UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM PSIQUIATRIA E CIÊNCIAS DO
COMPORTAMENTO

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**Influência da fluoxetina na microbiota intestinal de camundongos: análise
genômica e predição metabólica**

Porto Alegre

2021

CIP - Catalogação na Publicação

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Influência da fluoxetina na microbiota intestinal de camundongos: análise genômica e predição metabólica

Dissertação de Mestrado como requisito parcial à obtenção do título de Mestre em Psiquiatria pela Universidade Federal do Rio Grande do Sul.

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Porto Alegre

2021

AGRADECIMENTOS

À minha namorada Natalia, sem a qual este (e outros) projetos não teriam sido possíveis.

À minha família, que me apoiou mesmo sem ter muita certeza do que eu estava fazendo.

Aos meus orientadores Maria Paz e Francisco, pelas horas de dedicação e esforço.

Ao Pabulo, pela sua disposição em lidar com o intangível.

Aos demais colegas do Laboratório de Cronobiologia e Sono, em especial àqueles que me ajudaram em outros projetos.

RESUMO

Antidepressivos da classe dos inibidores seletivos da recaptação da serotonina (ISRS) como a fluoxetina têm recebido relatos consistentes quanto a sua influência na microbiota intestinal por meio de mecanismos complexos ainda em investigação. O funcionamento dessa interação tem recebido maior atenção da comunidade científica, tendo em vista sua potencial relação com o desenvolvimento de doenças psiquiátricas como a depressão. Considerando a complexidade na diversidade e na composição das comunidades bacterianas, uma melhor compreensão das rotas envolvidas nessa interação é uma perspectiva promissora. Porém ainda existem relativamente poucos estudos *in vivo* sobre o assunto. Com o objetivo de melhor avaliar o efeito deste antidepressivo na microbiota fecal de camundongos em sua composição e predição metabólica, foi realizada uma busca por estudos que contemplassem o uso de fluoxetina e a microbiota intestinal animal, cujos repositórios genômicos tenham sido gerados a partir de sequenciamento de alto rendimento por meio da Plataforma Illumina e disponibilizados de forma pública. Foi feito um processamento das sequências das amostras por meio de métodos de bioinformática, seguido de análises de alfa e beta diversidades, além de predição metabólica das comunidades microbianas. No estudo de Sun et al. (2019), 18 amostras fecais individuais de camundongos foram divididos em três grupos: controles + PBS (n = 6), animais não tratados expostos a estresse crônico imprevisível (CUMS + PBS) (n = 6) como modelo para depressão e um grupo similar exposto ao estresse mas tratado com fluoxetina (CUMS + fluoxetina) (n = 6). A análise dos dados a partir dos parâmetros do presente estudo sugere que os grupos apresentaram composições diversas da microbiota intestinal, onde 349 táxons bacterianos (OTU) foram identificados, com 10 filos e 93 gêneros. A análise linear discriminante (LDA) do tamanho do efeito (LEfSe) das alterações da microbiota intestinal mostrou que o filo bacteriano mais diferencialmente abundante foi Bacteroidetes (LDA 2,6), seguido por Proteobacteria (LDA 2,34) e Firmicutes (LDA 2.27). Com relação aos gêneros, os mais diferencialmente abundantes foram Muribaculaceae_unclassified (LDA 2.61), *Escherichia-Shigella* (LDA 2.43), *Parabacteroides* (LDA 2.36), *Lactobacillus* (LDA 2.24), *Subdoligranulum* (LDA 2.22), Lachnospiraceae_unclassified (LDA 2.19). A predição metabólica identificou 170 rotas metabólicas, e a análise LEfSe mostrou mudanças significativas em 166 delas. Diferenças estatísticas foram observadas principalmente para a sinalização Ras (LDA 8,89) e degradação de hidrocarbonetos aromáticos policíclicos (LDA 8,87). No estudo de Lyte et al. (2019), amostras fecais de grupos de camundongos expostos à fluoxetina (N = 10) e Controles saudáveis (N = 10), com fezes coletadas nos dias 0, 15 e 29 foram avaliados. A análise de composição identificou 10 filos e 118 gêneros, mas a composição não evidenciou diferenças significativas entre os grupos, considerando um escore LDA mínimo de 1,5 e um valor de p <0,05. Após a análise dos resultados do presente estudo, pode-se observar os efeitos da exposição ao estresse na redução da diversidade bacteriana e os potenciais efeitos da fluoxetina na amenização do processo de disbiose, bem como identificar potenciais rotas metabólicas de interesse para futuros estudos, como sinalização da Ras e degradação de hidrocarbonetos aromáticos policíclicos.

Palavras-chave: microbiota, fluoxetina, modelos animais, depressão, sequenciamento de alto rendimento, redes e vias metabólicas.

ABSTRACT

Selective serotonin reuptake inhibitor antidepressants, such as fluoxetine, have received consistent reports regarding their influence on the intestinal microbiota through complex mechanisms still under investigation. The functioning of this interaction has received greater attention from the scientific community, considering its potential relationship with the development of psychiatric illnesses such as depression. Considering the complexity in the diversity and composition of bacterial communities, a better understanding of the routes involved in this interaction is a promising perspective. However, there are still relatively few *in vivo* studies on the subject. In order to better assess the effect of this antidepressant on the fecal microbiota of mice in its composition and metabolic prediction, a search was carried out for studies that contemplated the use of fluoxetine and the animal intestinal microbiota, whose genomic repositories have been generated from sequencing through the Illumina Platform and made publicly available. Sample sequences were processed using bioinformatics methods, followed by alpha and beta diversity analyses, in addition to metabolic prediction of microbial communities. In the study by Sun et al. (2019), 18 individual mouse fecal samples were divided into three groups: controls + PBS (n=6), untreated animals exposed to unpredictable chronic stress (CUMS + PBS) (n=6) as a model for depression, and one group similar exposed to stress but treated with fluoxetine (CUMS + fluoxetine) (n=6). Data analysis from the parameters of the present study suggests that the groups presented different compositions of the intestinal microbiota, where 349 bacterial taxa (OTU) were identified, with 10 phyla and 93 genera. Linear discriminant analysis (LDA) of the effect size (LEfSe) of changes in the intestinal microbiota showed that the most differentially abundant bacterial phylum was Bacteroidetes (LDA 2.6), followed by Proteobacteria (LDA 2.34) and Firmicutes (LDA 2.27). Regarding genera, the most differentially abundant were Muribaculaceae_unclassified (LDA 2.61), Escherichia-Shigella (LDA 2.43), Parabacteroides (LDA 2.36), Lactobacillus (LDA 2.24), Subdoligranulum (LDA 2.22), Lachnospiraceae_unclassified (LDA 2.19). Metabolic prediction identified 170 metabolic pathways, and LEfSe analysis showed significant changes in 166 of them. Statistical differences were observed mainly for Ras signaling (LDA 8.89) and degradation of polycyclic aromatic hydrocarbons (LDA 8.87). In the study by Lyte et al. (2019), fecal samples from groups of mice exposed to fluoxetine (N = 10) and healthy controls (n=10) (days 0, 15, and 29) were evaluated. The composition analysis identified 10 phyla and 118 genera, but the composition did not show significant differences between the groups, considering a minimum LDA score of 1.5 and a p-value <0.05. After analyzing the results of this study, one can observe the effects of exposure to stress in reducing bacterial diversity and the potential effects of fluoxetine in alleviating the process of dysbiosis, as well as identifying potential metabolic routes of interest for future studies, such as Ras signaling and degradation of polycyclic aromatic hydrocarbons.

Keywords: microbiota, fluoxetine, depression animal models, high-throughput nucleotide sequencing, metabolic networks and pathways.

SUMÁRIO

1. INTRODUÇÃO

Microbiota é definida como uma comunidade de microrganismos que habitam um dado ambiente. O termo é mais comumente usado na área da saúde em referência às populações microbianas nativas do corpo humano ou de animais, formada desde o primeiro contato com o ambiente externo após o nascimento. Em mamíferos, este momento se dá na saída do útero, após o rompimento da placenta, trazendo contaminação a um espaço até então estéril. A colonização do recém nascido pelos microorganismos presentes no canal vaginal materno vem se mostrando um importante componente no desenvolvimento inicial do sistema imune (1). Mesmo que o contato com microorganismos venha inevitavelmente ocorrer em outras formas de nascimento, com o parto cesáreo, poderá haver uma perturbação no estabelecimento e desenvolvimento da microbiota na criança (2). Esta convivência tão precoce entre microorganismos e hospedeiro ilustra a importância da relação entre ambos para o bem estar e o desenvolvimento (3).

Entre os habitats disponíveis no corpo, o trato digestivo é aquele com a maior população de microorganismos, tendo em vista sua conexão com o ambiente externo e a vasta quantidade de nutrientes disponíveis (4). Embora seja composta por diversos tipos de microorganismos, como vírus, fungos e protozoários, quando nos referimos à microbiota intestinal usualmente falamos sobre bactérias, cujo número amplamente supera os demais (5). Uma estimativa recente sugere que a proporção de células humanas e bacterianas em um hospedeiro é de aproximadamente 1: 1 (6). Esta vasta população parece exercer um importante papel na saúde do hospedeiro, mediando processo e vias metabólicas que vão além de sua influência local (3). Em condições normais, a regulação cruzada entre o hospedeiro e a microbiota intestinal

cria um equilíbrio homeostático de bactérias para que o trato gastrointestinal permaneça saudável e livre de crescimento excessivo de organismos potencialmente patogênicos.

Da mesma forma que uma microbiota estável parece estar ligada à saúde, mudanças em sua composição podem causar situações de desequilíbrio que contribuem com a fisiopatologia de doenças, um conceito conhecido como disbiose (7). Inicialmente, pesquisas ligadas à microbiota estavam relacionadas a enfermidades locais do intestino, como Doença de Crohn e Doença inflamatória intestinal (8,9). No entanto, cada vez mais foram observadas situações de alteração significativa da microbiota intestinal em patologias que iam além de sua aparente influência local, como Doença de Alzheimer (10), Parkinson (11), esquizofrenia (12) e depressão (13).

A depressão é atualmente a principal causa de prejuízo funcional relacionado à saúde no mundo, afetando cerca de 300 milhões de pessoas (14). Seu curso insidioso, caráter multifatorial e resposta variável ao tratamento tornam de suma importância a detecção precoce e o adequado manejo para um melhor prognóstico. Da mesma forma, a elucidação de seus mecanismos fisiopatológicos é uma linha de pesquisa de suma importância.

A primeira linha de tratamento medicamentoso envolve o uso de compostos da classe dos inibidores seletivos da recaptação da serotonina (ISRS), cuja principal mecanismo de ação eleva os níveis de serotonina no cérebro, inibindo sua reabsorção pelos neurônios na fenda sináptica. Fluoxetina foi a primeira medicação descoberta desta classe (15), sendo amplamente utilizada em pesquisas e na prática clínica. Explorando estudos focados na microbiota intestinal e em sua interação com a fluoxetina e outros ISRS, foi possível postular quais outras vias poderiam estar

envolvidas na gênese de sintomas depressivos e ansiosos. O conceito da existência de um eixo intestino-cérebro, uma rota bidirecional de comunicação entre o sistema nervoso central e entérico, tem ajudado a compreender melhor as diferentes maneiras pelas quais a microbiota intestinal pode influenciar processos neuropsiquiátricos e por sua vez ser influenciada por eles (16).

Diferentes vias foram propostas e observadas como fazendo parte deste eixo, incluindo o sistema imunológico, o metabolismo do triptofano, o nervo vago e o sistema nervoso entérico. Além destes, não só a composição taxonômica da microbiota, mas a análise dos metabólitos influenciados por estas populações parecem ter um importante papel, levando à proposta de expansão para um eixo microbiota-intestino-cérebro (17).

Estudos mostraram a influência de fluoxetina e outros psicofármacos em modificações na variedade e composição das bactérias intestinais, bem como em sua metabolômica, o conjunto de metabólitos produzidos e modificados pelos mesmos (18). Desta forma, análises metabólicas vêm sendo cada vez mais utilizadas neste campo de pesquisa para o entendimento dos mecanismos relacionados ao estresse e à depressão (19).

Tendo em vista a existência de inúmeras funções metabólicas e interações entre genes, é de grande valia aumentar as bases de conhecimento sobre os mesmos para uma compreensão mais ampla de suas funções implícitas. Torna-se necessário não apenas avaliar as alterações composicionais do microbioma intestinal, mas também estabelecer predições de rotas metabólicas dos microrganismos associados a tais condições. Estas análises complexas podem ser realizadas através do uso de novas tecnologias, com o emprego de métodos de sequenciamento de alto rendimento, que permitem analisar sequências gênicas empregando processos

paralelos em grande escala para execuções mais rápidas e com melhor custo-benefício em comparação a métodos mais antigos (20).

Modelos animais são de grande utilidade na compreensão de modificações na microbiota, permitindo intervenções que não são possíveis em humanos para estudar o papel causal da microbiota intestinal na saúde e na doença. Fontes de variações, como dietas e condições de alojamento, são geralmente controladas em experimentos, limitando influências indesejadas do ambiente externo à microbiota intestinal (21).

Embora não seja possível reproduzir fielmente a complexa gama de estímulos e estressores ligados à rotina humana, modelos de estresse crônico brando e imprevisível (*chronic unpredictable mild stress*, ou CUMS) são capazes de reproduzir respostas análogas em roedores. Tais protocolos de exposição ao estresse já vem sendo utilizados em pesquisas composição e metabolômica de populações bacterianas (22).

Trabalhos recentes empregaram modelos animais, em diferentes condições de controle, na pesquisa da microbiota intestinal ligada ao uso de antidepressivos e à exposição ao estresse. Fung et al. 2019 (23) descreveu a colonização bacteriana no intestino quando influenciada pelo uso de fluoxetina, apoiando a ideia de que bactérias da microbiota sinalizam bidirecionalmente com o sistema serotonérgico do hospedeiro para promover um ambiente de equilíbrio no intestino. Lukić et al., 2019 (24) demonstrou que grupos de animais expostos a diferentes antidepressivos, incluindo fluoxetina, tiveram a composições de suas microbiotas alteradas, além de implicar a suplementação com a bactéria *Ruminococcus flavefaciens* na redução de comportamentos depressivos. Tian et al., 2019 (25) observou que a suplementação com uma subespécie de *Bifidobacterium longum* pode prevenir o início de

comportamento depressivo em modelos de estresse crônico, utilizando um grupo controle tratado com fluoxetina. Siopi et al., 2020 (26) conduziu um estudo em que a microbiota de camundongos expostos ao modelo de estresse crônico brando e imprevisível foi transferida para receptores saudáveis, revelando que as alterações induzidas pelo estresse estão envolvidas na patogênese de sintomas depressivos e minimizam a eficácia do tratamento com fluoxetina por meio de alterações na via serotonérgica do metabolismo do triptofano.

Entre os estudos com modelos animais envolvendo microbiota e fluoxetina, dois se destacaram por ter disponibilizado suas bases de dados genômicas de forma pública através da *National Center for Biotechnology Information* (NCBI), depositadas no *Sequence Read Archive* (SRA). Sun et al. 2019 (27) investigou as mudanças na microbiota intestinal de camundongos em um modelo de depressão induzida por estresse crônico brando e imprevisível, comparado a um modelo similar tratado com fluoxetina, revelando que a mesma levou à redução da disbiose induzida pelos estressores. Lyte et al., 2019 (28) seguiu dois grupos de animais saudáveis, um dos quais exposto à fluoxetina, com coletas de fezes em três momentos dentro de um mês, concluindo que houve uma alteração significativa, dependente do tempo, nas comunidades microbianas, acompanhadas por mudanças no peso corporal.

Como uma área de estudo relativamente recente, ainda existem poucos estudos *in vivo* que tenham realizado a análise da microbiota intestinal expostas à fluoxetina ou outros antidepressivos, abordando não só sua composição, mas seu perfil metabólico. A análise secundária dos dados disponibilizados pelos pesquisadores de forma livre e gratuita permitirá aprofundar a investigação com os materiais já coletados.

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2. OBJETIVOS

O objetivo geral do estudo foi avaliar o efeito da fluoxetina na micrpbiota fecal de camundongos por meio de uma análise secundária de dados públicos disponíveis na literatura.

Os objetivos específicos foram:

a) selecionar artigos que versem sobre o efeito de fluoxetina na microbiota fecal de camundongos por meio de sequenciamento de alto rendimento utilizando a Plataforma Illumina.;

b) acessar sequências de ácidos nucleicos geradas a partir de sequenciamento de alto rendimento, por meio da Plataforma Illumina, e depositadas em bancos públicos de sequências genômicas;

c) realizar processamento das sequências por meio de métodos de bioinformática;

d) realizar análise da diversidade beta e alfa das comunidades microbianas de amostras de fezes de camundongos expostos ou não à fluoxetina;

e) realizar análise de predição metabólica das comunidades microbianas de amostras de fezes de camundongos expostos ou não à fluoxetina.

3. ARTIGO CIENTÍFICO

Influence of Fluoxetine on the intestinal microbiota of mice: genomic analysis and metabolic prediction

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ABSTRACT

Antidepressants of the selective serotonin reuptake inhibitor (SSRI) class such as fluoxetine have been consistently reported to influence gut microbiota through complex mechanisms yet in research. The inner workings of this interaction have received greater attention from the scientific community, considering its potential connection with the development of psychiatric illnesses such as depression. Considering the complexity in diversity and composition of bacterial communities, a better comprehension of the diverse pathways involved in this interaction is a promising perspective, yet there are still relatively few *in vivo* studies on this subject. As the number of studied individuals is limited, the joined analysis of existing data might add to the robustness for more precise conclusions. In order to better assess the effect of this antidepressant on the fecal microbiota of mice in its composition and metabolic prediction, a search was carried out for studies that contemplated the use of fluoxetine and the animal intestinal microbiota, and whose genomic repositories were publicly available. Next, an *in silico* analysis of the structure and function of microbial communities was performed using this open data. The study allowed us to observe the effects of exposure to stress in reducing bacterial diversity and the potential effects of fluoxetine in alleviating the dysbiosis process and identifying potential metabolic pathways of interest for future studies.

Keywords: microbiota, fluoxetine, depression, animal models, high throughput nucleotide sequencing, metabolic networks and pathways.

INTRODUCTION

Human beings are inhabited by a varied population of microorganisms in different places in their body, called in its entirety the microbiota (1). By evolving in parallel with humanity, these commensals have developed a mutually beneficial relationship that is closely linked to the well-being of the host (2). Of particular interest, the native gut microbiota and metabolome appear to influence locally and the central nervous system (3). Dysbiosis, an imbalance in a healthy microbiota's composition and diversity, has also been shown to impact the host's mental health (4).

Depressive symptoms are a significant health problem which the primary pharmacotherapeutic approach is through the use of antidepressants, especially those of the selective serotonin reuptake inhibitor class (5). The mechanisms of regulation of the enteric metabolism of serotonin are still not clearly understood, but growing evidence presents the gut-brain axis as a possible key in the genesis of these symptoms (6). This bidirectional route between central and enteric nervous systems revolves around multiple pathways, and microbial composition and metabolites seem essential in regulating this communication (7).

The influence of stress on the microbiota has been known for over 40 years (8). In recent decades, the impact of antidepressants on their composition has been investigated by analyzing their populations by high-throughput sequencing methods. Recent studies point to a possible role of the intestinal microbiota associated with anxious and depressive behaviors (9,10). It has also been observed that psychotropic medications, including antidepressants, can influence *in vivo* the composition of this same microbiota (11,12).

As the first antidepressant of the class of selective serotonin reuptake inhibitors, fluoxetine is widely recognized as a drug of significant impact in depression treatment and research (13). Aside from its primary mechanism of modulating serotonin reabsorption in the synaptic gap, in recent years, its role as a modulator of the intestinal microbiota and metabolome has been better observed (14). The influence of fluoxetine on the variability and abundance of different intestinal bacterial strains could be observed in mice (15), also demonstrating that it can reduce dysbiosis in models of depression in these animals (16).

This article aims to further describe the effects of fluoxetine on the fecal microbiota of animal models through a review of current studies and secondary analysis of data made available in public repositories, focused not only on the community composition but also on the predicted metabolic profiles.

MATERIAL AND METHODS

This study was carried out at Hospital de Clínicas de Porto Alegre, within the Laboratory of Chronobiology and Sleep research facilities and the Experimental Research Center. The research protocol was approved by the GPPG, Hospital de Clínicas de Porto Alegre (Porto Alegre, RS) (Protocol Number 2021-0116), and also by the Research Board, Universidade Federal do Rio Grande do Sul (Protocol Number 40465).

Firstly, it comprised a review assessing the currently available studies on the subject, with a search method utilizing items 5 through 8 of the "PRISMA for Scoping Reviews" checklist (17). Papers were selected among studies with cohorts of animal models exposed or not to the antidepressant fluoxetine that describe models of

depression or employed healthy animals, that had their genomic repositories sequenced using the Illumina platform, and whose sequence data obtained were publicly available, excluding those not meeting these criteria.

The review search was carried out with the terms "fluoxetine" AND "microbiome", utilizing PUBMED, SCOPUS, Web of Science, and EMBASE databases. Of the 142 matches, 8 were excluded after eliminating duplicates. Of the remaining 134 articles, 123 were excluded as they were not cohort studies with targeted populations. Of these, 1 had the analysis of blood samples as an outcome, and three did not have their text available. Of the eight studies analyzing the fecal microbiota of mouse feces, 2 had publicly available genomic sequencing data: Sun et al., 2019 (Biosample Code PRJNA486701) (16) and Lyte et al., 2019 (Biosample Code SRP145610) (18). An *in silico* analysis of the structure and function of microbial communities was then conducted utilizing the genomic repositories of these two studies. The flowchart for the article selection is detailed in **Figure 1**.

Obtaining database sequences

The two sets of data to be analyzed in this project are deposited in the public repository of NGS data maintained by the NCBI "Short Read Archive" with the access codes PRJNA486701 (16) and SRP145610 (18). The data made available in the SRA database is compressed in a specific file format called ".sra". These will be downloaded and converted to FASTA format using bioinformatics tools and analyzed as described below.

Sequence data obtained from the RAS was processed using the Mothur v.1.41.1 software (19), following an adapted version of the pipeline (20), also

described in Moraes et al. (2020) (21). After the initial filtering and trimming steps, the other sequences were grouped into Operational Taxonomic Units (OTUs) based on 99% similarity and classified with the SILVA v132 reference database with 97% similarity (22). The resulting sequences, frequencies, and taxonomic attributions were converted into a biom format file for microbial communities analysis. Subsequent analysis of the sequence dataset was performed in R v.3.6.1 (using vegan, phyloseq, ggplot2, and microbiotaAnalystR packages) or QIIME v.1.9.1.

Compositional analysis of bacterial communities

OTU abundances were used to calculate alpha diversity metrics, including species richness, Shannon diversity index, and Simpson diversity index. To compare significant differences between bacterial communities (i.e., beta diversity), principal coordinate analysis (PCoA) was performed. A matrix using phylogenetic and non-phylogenetic metrics was calculated for each pair of samples. The distances were transformed into points in space with dimensions one less than the number of samples. Clustering methods based on Bray-Curtis dissimilarity and Pearson correlation were performed to compare other differences between microbial communities. The results of the hierarchical grouping were visualized using heatmaps and dendrograms. The linear discriminant effect size (LEfSe) method was used to analyze differentially abundant taxa (23). Differences were considered significant with an "LDA score" of 1.5 and a p-value <0.05.

Metabolic prediction

The functional predictive gene profiling was based on 16S rRNA gene sequencing data using Piphillin (24) described according to the updated Kyoto Encyclopedia of Genes and Genomes (KEGG) database for systematic analysis of gene functions (25), and a 97% confidence cutoff value. Piphillin uses direct nearest-neighbor correspondence between 16S rRNA amplicons and genomes to predict the represented genomes. The comparison of the functional profiles of each population was performed with a PERMANOVA analysis. The differentially abundant traits were determined using the linear discriminant analysis (LDA) effect size (LefSe). The Benjamini-Hochberg adjusted p-value was calculated to control the false discovery rate (FDR). The KEGG groups were considered differentially abundant, satisfying a corrected p-value (FDR) of 0.05.

RESULTS

Both studies were analyzed utilizing the methods described above.

Gut bacterial community diversity when exposed to stress and Fluoxetine treatment [Sun et al. (2019) analysis].

The microbiota profiles from 18 individual fecal samples from mice were divided into three groups: Controls + PBS (n=6), untreated animals exposed to chronic unpredictable mild stress (CUMS + PBS) (n=6) as a model for depression, and a similarly stress-exposed group treated with Fluoxetine (CUMS + fluoxetine) (n=6) were analyzed (16).

Analyzing alpha diversity of bacterial communities evidenced varying degrees through the Shannon index **(Figure 2A)**, with higher diversity in the Control group and lower in the untreated CUMS + PBS group. The fluoxetine group had a closer value for the Shannon Index to the healthy controls, in contrast with untreated animals exposed to the stress model, showing the effect of fluoxetine in controlling the reduction of bacterial diversity.

Principal coordinates analysis (PCoA) was used to determine the clustering of samples and to understand better similarities and differences between the bacterial community structures of the three groups **(Figure 2)**. Bray–Curtis **(Figure 2B)** and weighted UniFrac **(Figure 2C)** distances were used to conduct simultaneous comparisons of all microbial communities, using the Bray–Curtis metric, samples clustered according to each group ($P < 0.05$, ANOSIM). While both clusters show significant differences, weighted UniFrac analysis shows the two CUMS groups closer to each other.

Unweighted Pair Group Method with Arithmetic mean revealed a noticeable distinction among groups **(Supplementary Figure 1)**, with the CUMS+Fluoxetine having a clustering similarity with the CUMS + PBS group. Both groups were not similar to the Control group. Both alpha and beta diversity suggested a closer pattern between both CUMS groups. Exposure to fluoxetine, although not undoing the apparent change in a bacterial community that might be attributed to stress, does partially offset it.

The study groups displayed diverse gut microbiota compositions **(Figure 3A and B)**. Three hundred forty-nine bacterial taxa (OTU) were identified, with 10 phyla and 93 genera. Linear discriminant analysis (LDA) effect size (LEfSe) analysis of gut microbiota changes with an LDA score of 1.5 and a p-value <0.05 showed that the

most differentially abundant bacterial phylum **(Figure 3C and D)** was *Bacteroidetes* (LDA 2,6), followed by *Proteobacteria* (LDA 2.34) and *Firmicutes* (LDA 2.27), with still significant *Epsilonbacteraeota* (LDA 1.53) and *Actinobacteria* (LDA 1.52). *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were more enriched in the CUMS + PBS group when compared to Controls, the opposite occurring with *Bacteroidetes* and *Epsilonbacteraeota*. Notably, all phyla demonstrated a more stable profile in the CUMS + Fluoxetine samples. Regarding genera, the most differentially abundant were *Muribaculaceae_unclassified* (LDA 2.61), *Escherichia-Shigella* (LDA 2.43), *Parabacteroides* (LDA 2.36), *Lactobacillus* (LDA 2.24), *Subdoligranulum* (LDA 2.22), *Lachnospiraceae_unclassified* (LDA 2.19).

Metabolic prediction from samples bacterial gene profile identified 170 metabolic pathways modules, encompassing Metabolism, Genetic Information Processing, Environmental Information Processing, and Cellular Processes. LEfSe analysis of distinct pathways showed significant changes in 166 (LDA 1,5, corrected p-value of 0,05) **(Supplementary Table 2)**. The ones with the highest differences between study groups were Ras signaling pathway (LDA 8,89), Polycyclic aromatic hydrocarbon degradation (LDA 8,87), Caffeine metabolism (LDA 4,71), and Furfural degradation (LDA 3,94). Additional pathways with significant LDA values are in **Supplementary Figure 2.**

Gut bacterial community diversity in a longitudinal study with fluoxetine and controls [*Lyte et al.* (2019) analysis].

The Lyte 2019 (18) study followed a group of mice exposed to Fluoxetine (N=10) versus healthy Controls (N=10), with feces collected in days 0, 15, and 29, totalizing 60 fecal samples. No stress models were involved.

Comparing the sum of samples in both groups using alpha diversity analysis, Fluoxetine and Control did not show a significant difference. **(Figure 4A).**

Bray–Curtis PCoA had an apparent superposition, whether analyzing the total pool of Fluoxetine and Control samples (p=0.007) **(Figure 4B)**, the total follow-up in different collecting days (p=0.1) **(Figure 4C),** or the follow up of each separate Fluoxetine and Control groups in different collecting days (p=0.01) **(Figure 4D)**, without significant difference between them.

The composition analysis identified 10 phyla and 118 genera **(Figure 5A and B)**, with *Bacteroidetes* and *Firmicutes* as the most abundant phyla. As analyzed, the composition did not evidence significant differences between the groups. LEfSe analysis found low LDA scores **(Supplementary Table 3).** Considering an LDA score of 1.5 and a p-value <0.05, no significant differences were found.

DISCUSSION

Animal models have offered an important parallel for the research on mechanisms of depression and antidepressant medication. As human subjects are exposed to complex contexts of stressors, the existence of a controlled environment for animal models in microbiota research allows for a comprehensive analysis before it can be applied adequately to treatment. In the form of shared, publicly available gene repositories, joint analysis of existing data adds to the robustness sought for more precise conclusions in a field where the number of studied individuals is still limited (26,27). Meta-analysis of microbiota diversity through public repositories has already been applied in other studies with animals (28,29) but might have a more significant impact in health studies in the future. Approaches like bacterial growth rate and

genome-scale metabolic modeling might play an essential part in understanding the contribution of the gut microbiota to depression etiology, but heterogeneity was a limiting factor regarding the selection of studies. A broad pool of methods, doses, and antidepressants is used, making the comparison between them less valid and meta-analysis impossible (30). On the other hand, it also encourages the use of publicly available data that can be used to deepen current discussion, utilizing more advanced and refined analysis methods than the ones available when the studies were conducted.

Although not directly comparable due to distinct methodologies, the presently selected studies were still able to shed light on the behavior of gut bacteria in different conditions of exposure and treatment. Sun et al. (2019) (16) showed that both groups of mice exposed to chronic stress had a lower diversity in bacterial taxa when compared to controls. Lower diversity is the most common disruption in microbiota composition linked to dysbiosis (31). A higher gut bacterial diversity is considered beneficial for individual health, while decreased microbiota richness is associated not only with clinical conditions, such as irritable bowel disease and obesity (32,33), but also neuropsychiatric conditions, like Alzheimer (34), Parkinson (35), schizophrenia (36) and depression (37).

The analysis of data extracted from Lyte et al. (2019) (18) showed no significant difference in composition between the followed control and fluoxetine groups, even though fluoxetine and other antidepressants have been observed to differentially influence the composition of gut microbiota *in vivo* (11). Lacking a stress protocol, alterations in gut microbiota were not so severe, which points to stress as a relevant factor in those changes (38).

The link between the use of fluoxetine in modulating bacterial colonization was observed in different studies (15,39). Interestingly, not only antidepressants but also the regulation of microbiota through the use of bacterial genera supplementation with *Ruminococcus flavefaciens* can lead to an improvement of depressive behavior in mice (40), exemplifying the bidirectional connection between gut and brain.

The healthy gut microbiota composition is still a matter of debate, as the bacterial profile varies in individuals according to factors like age, diet, established routine, and use of medications (41). Alterations in scenarios of stress or illness tend to cause changes in an individual's normal microbiota. A decrease in *Bacteroidetes* has been observed in previous studies where animals were exposed to social stressors (42).

The present study shows that *Firmicutes* and *Bacteroidetes* were the two main phyla representatives of gut microbiota composition, an established typical pattern for both mice and humans (43,44). In the analysis performed by Sun *et al.* (2019), *Bacteroidetes* and *Epsilonbacteraeota* had lower abundance in CUMS + PBS, while *Firmicutes*, *Proteobacteria*, and *Actinobacteria* had their abundances increased. For the bacterial genera analysis, the same situation met a low abundance of *Muribaculacea* and a higher abundance of *Escherichia-Shigella* and *Lactobacillus*. Changes in composition have been previously reported in the literature for groups exposed to stress and antidepressants. However, as the results on taxa diversity change in the reports from the literature (30), there is no definitive archetype of microbiota in depression.

As in previous studies, pathways related to lipid and amino acid metabolism were enriched, such as for tryptophan metabolism (45). Changes in alanine, aspartate, and glutamate metabolism have already been reported in other studies with

antidepressants (46,47). Our study's composition and metabolic prediction analysis did not find significant changes in the Lyte 2019 data (18), but Sun 2019 (16) presented interesting pathways where further studies might focus.

Ras signaling showed itself as the most activated pathway in the fluoxetine group. It is linked to cell growth and differentiation and neuroplasticity, an important element in the pathophysiology of depression (48). It is suggested that antidepressant action might be partially due to stimulating adult neurogenesis, elevating proliferation of precursor cells, and increasing survival of immature neurons (49). The Ras pathway also seems to relate to adult neurogenesis in the dentate gyrus, a hippocampal structure thought to play a role in depression (50). There is evidence that fluoxetine increases the rate of neurogenesis and enhances neuroplasticity (51), contributing to its antidepressant effect. Its decreased signaling has been observed in the post-mortem prefrontal cortex and hippocampus of suicide patients with major depressive disorder (52).

The polycyclic aromatic hydrocarbon (PAH) degradation pathway had increased activation in both CUMS + Fluoxetine and CUMS + PBS groups, higher in the latter. This group of compounds can be commonly found in air pollution and smoking (53). A recent cross-sectional study dosed PAH metabolites in the urine of adult subjects and reported a positive association with those metabolites and depressive symptoms in females. (54). Exposure to PAH has also been correlated to ADHD symptoms and reduced caudate nucleus volume in children (55). In rodents, exposure could cause pathophysiological changes such as loss of neuronal activity and synaptic plasticity (56). No studies were found regarding PAH and Fluoxetine.

Diversity analysis of gut bacteria seems of great importance in the current scenario where microbiota alterations, representing the concept of dysbiosis, are

regarded as more influential to an individual's health than bacterial profile correctly (30). Lefse and LDA analysis can show that these variations can lead to microbial composition and metabolic pathways and be essential tools for future studies.

CONCLUSIONS

Exposure to a depression model like CUMS might play an essential role in reducing diversity in gut bacteria, leading to dysbiosis and consequent disruption of metabolic pathways maintained by a healthy microbiota. The dysbiosis caused by stress seems to be affected by fluoxetine, which, if not completely reverting those changes, partially offsets them. A better understanding is needed about what mechanisms can cause dysbiosis, how this process can influence hosts' physical and mental health, and how fluoxetine might influence positive outcomes. Metabolomics is a promising field for future research on how selective serotonin reuptake inhibitors can exert therapeutic effects beyond the central nervous system and how the bacteria influence those pathways.

Furthermore, it was possible to analyze public databases in microbiota and predicted metabolome research applied to mental health and depression. It is a helpful tool once more studies can be carried out as the collection of data increases. It allows for applying current analysis techniques that could not be available when the first experiment was carried out.

Funding Information

This study was partly financed by the Brazilian national funding agency, Coordenação de Aperfeiçoamento de Pessoal de Nıvel Superior – Brasil (CAPES).

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Acknowledgement

Authors would like to thank to Centro de Experimentação Animal and Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre (HCPA, Porto Alegre, Brazil) for technical support and for the research facilities.

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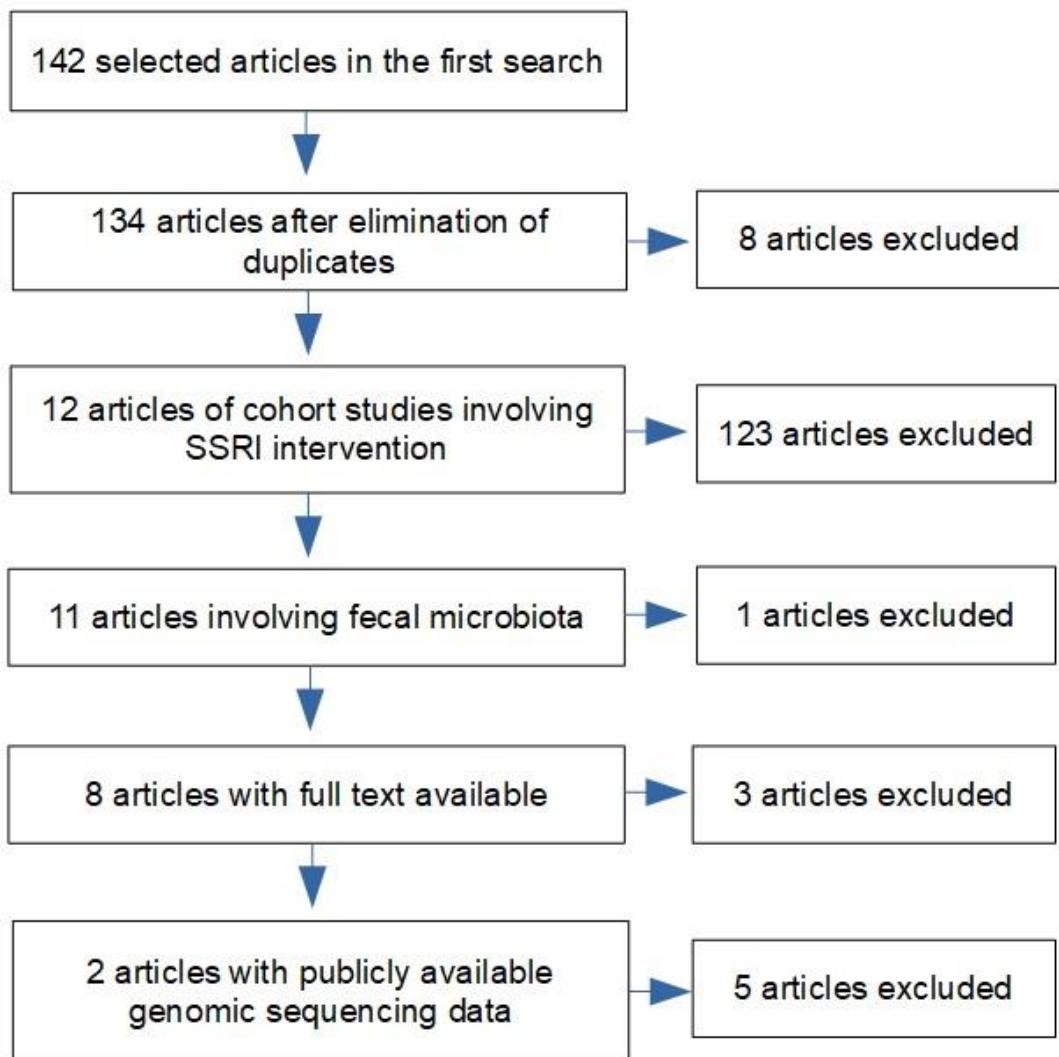
Figure 1: Article selection flowchart.
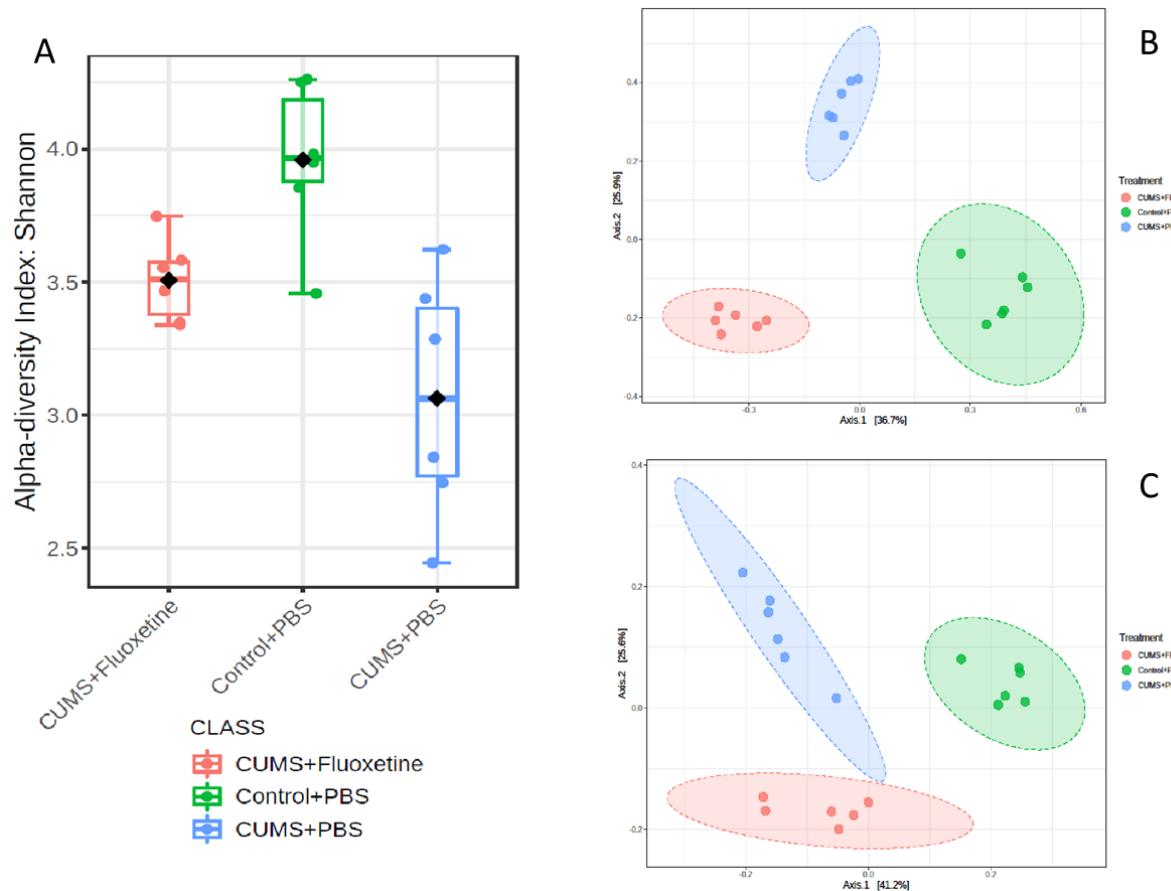
Figure 2: Gut bacterial community diversity, from Sun *et al.* (2019) study, when exposed to treated and untreated stress models. (A) Shannon index shows the bacterial alpha-diversity in 3 groups (CUMS+Fluoxetine, Control+PBS, CUMS+PBS), where fluoxetine was able to control the bacterial diversity. Principal coordinate analysis (PCoA) plot with Bray-Curtis (B) and Weighted Unifrac (C) dissimilarity shows the difference between the groups. Although all clusters show significant differences between the groups, weighted analysis shows them as less apart.

Figure 3: Differential abundance and composition of bacterial population described in phyla and genera, from the Sun *et al.* (2019) study. Relative abundance of intestinal bacteria when exposed to fluoxetine and different controls, according to phyla (A) and genera (B). In both stress-exposed groups (CUMS + PBS and CUMS + Fluoxetine), there was a higher prevalence of *Firmicutes* and *Proteobacteria* and a lower prevalence of *Bacteroidetes*. Linear discriminant analysis effect size (LEfSe) analysis of the degree of consistent difference (LDA score of 1.5 and p value <0.05) in phyla **(C)** and genera **(D)**.
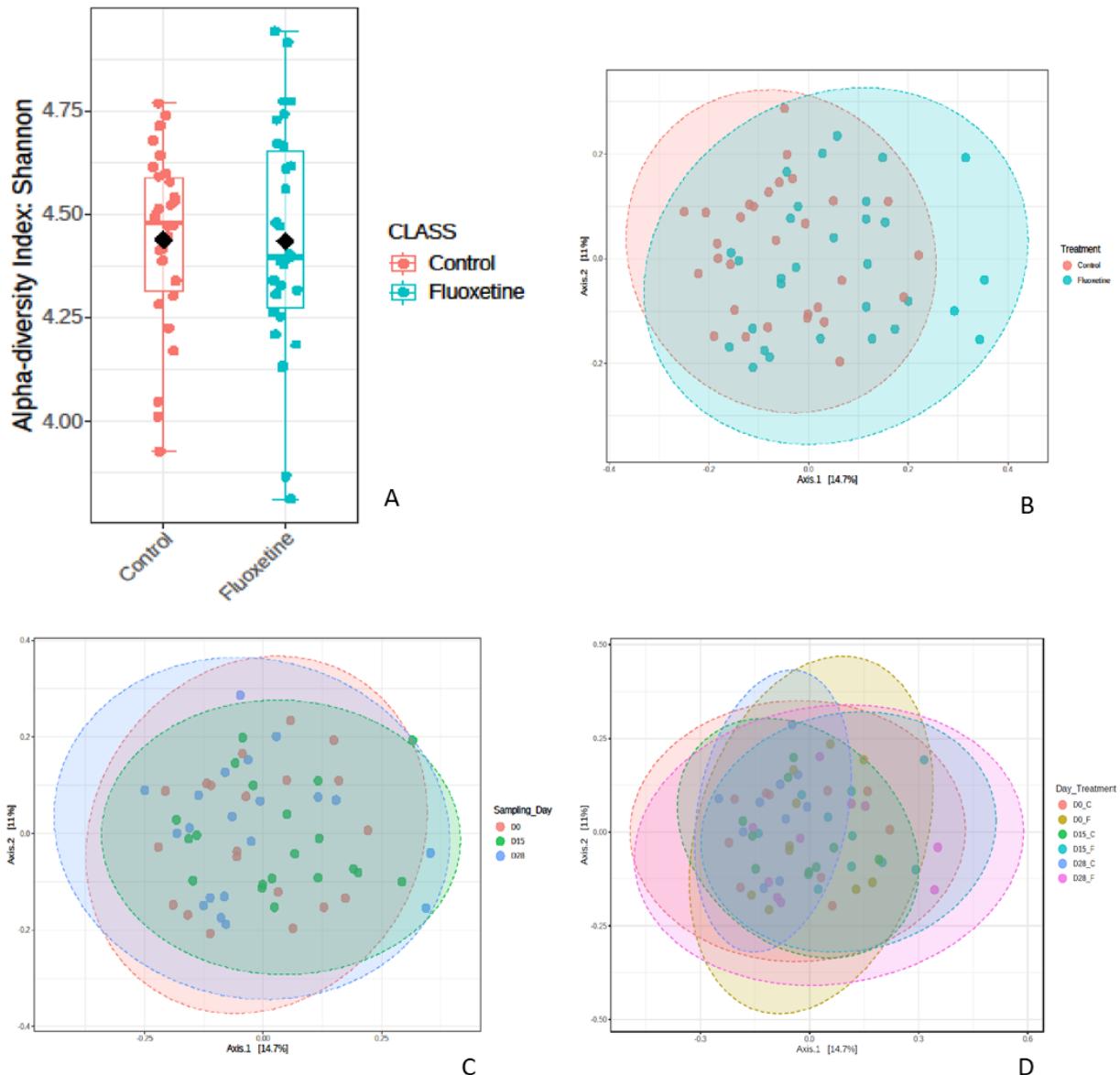
Figure 4: Gut bacterial community diversity, from Lyte *et al.* (2019) study, when exposed to treated and untreated stress models. (A) Shannon index did not show bacterial diversity in 2 groups (Fluoxetine and Control). Principal coordinate analysis (PCoA) plot with Bray-Curtis (B) and Weighted Unifrac (C) dissimilarity did not show the difference between the groups.
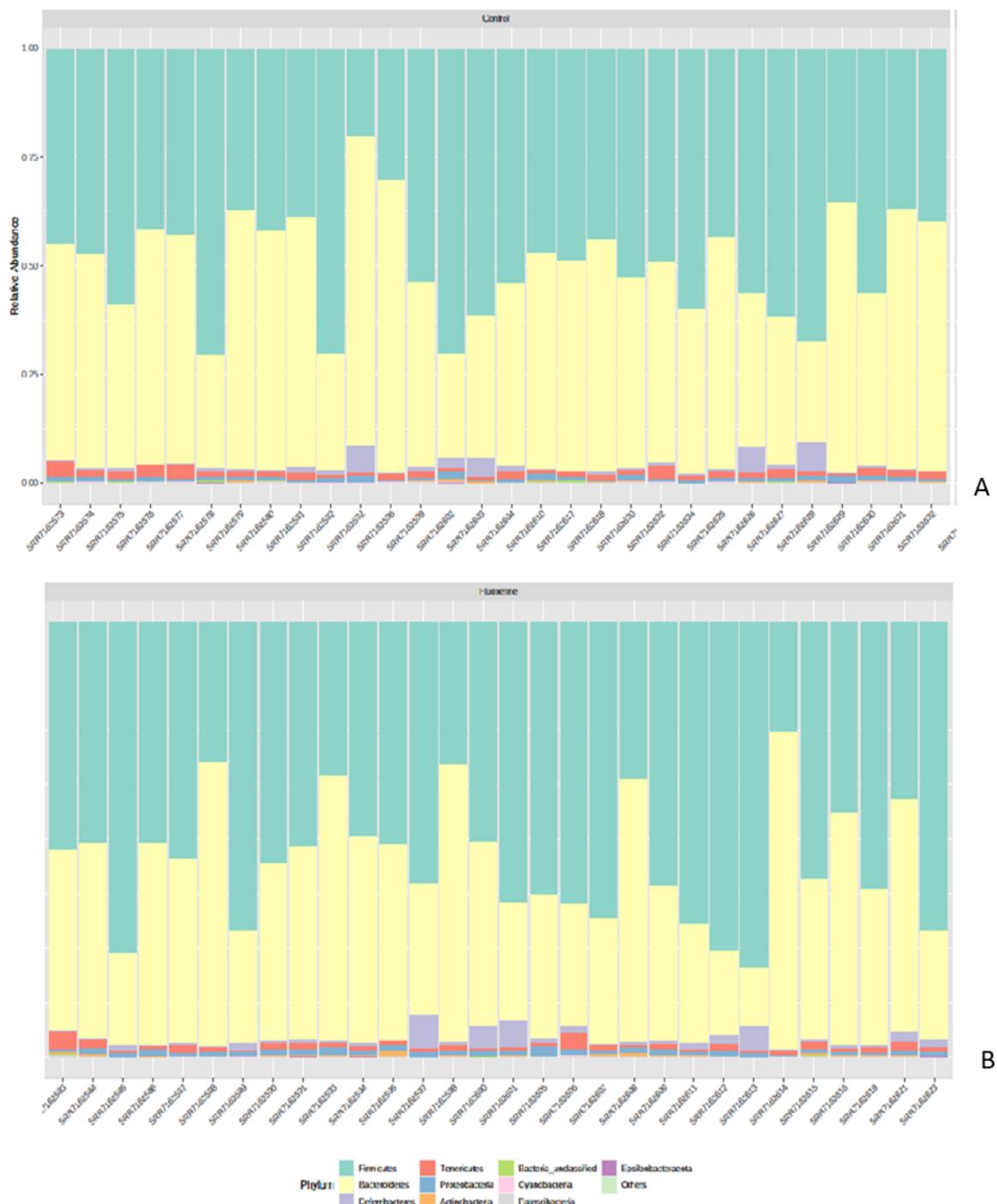
Figure 5: Lyte *et al.* (2019) study groups of controls (A) and fluoxetine according to phyla (B) and genera (C) did not present significant differences in composition.

Figure 5: Lyte *et al.* (2019) study groups of controls (A) and fluoxetine according to phyla (B) and genera (C) did not present significant differences in composition.

FIGURES SUBTITLES

Figure 1: Article selection flowchart.

Figure 2: Gut bacterial community diversity, from Sun, 2019 study, when exposed to treated and untreated stress models. (A) Shannon index shows the bacterial alpha-diversity in 3 groups (CUMS+Fluoxetine, Control+PBS, CUMS+PBS), where fluoxetine was able to control the bacterial diversity. Principal coordinate analysis (PCoA) plot with Bray-Curtis (B) and Weighted Unifrac (C) dissimilarity shows the difference between the groups. Although all clusters show significant differences between the groups, weighted analysis shows them as less apart.

Figure 3: Differential abundance and composition of bacterial population described in phyla and genera, from the Sun, 2019 study. Relative abundance of intestinal bacteria when exposed to fluoxetine and different controls, according to phyla (A) and genera (B). In both stress-exposed groups (CUMS + PBS and CUMS + Fluoxetine), there was a higher prevalence of *Firmicutes* and *Proteobacteria* and a lower prevalence of *Bacteroidetes*. Linear discriminant analysis effect size (LEfSe) analysis of the degree of consistent difference (LDA score of 1.5 and p value <0.05) in phyla **(C)** and genera **(D)**.

Figure 4: Gut bacterial community diversity, from Lyte, 2019 study, when exposed to treated and untreated stress models. (A) Shannon index did not show bacterial diversity in 2 groups (Fluoxetine and Control). Principal coordinate analysis (PCoA) plot with Bray-Curtis (B) and Weighted Unifrac (C) dissimilarity did not show the difference between the groups.

Figure 5: Lyte *et al.* (2019) study groups of controls (A) and fluoxetine according to phyla (B) and genera (C) did not present significant differences in composition.

SUPPLEMENTARY FIGURES
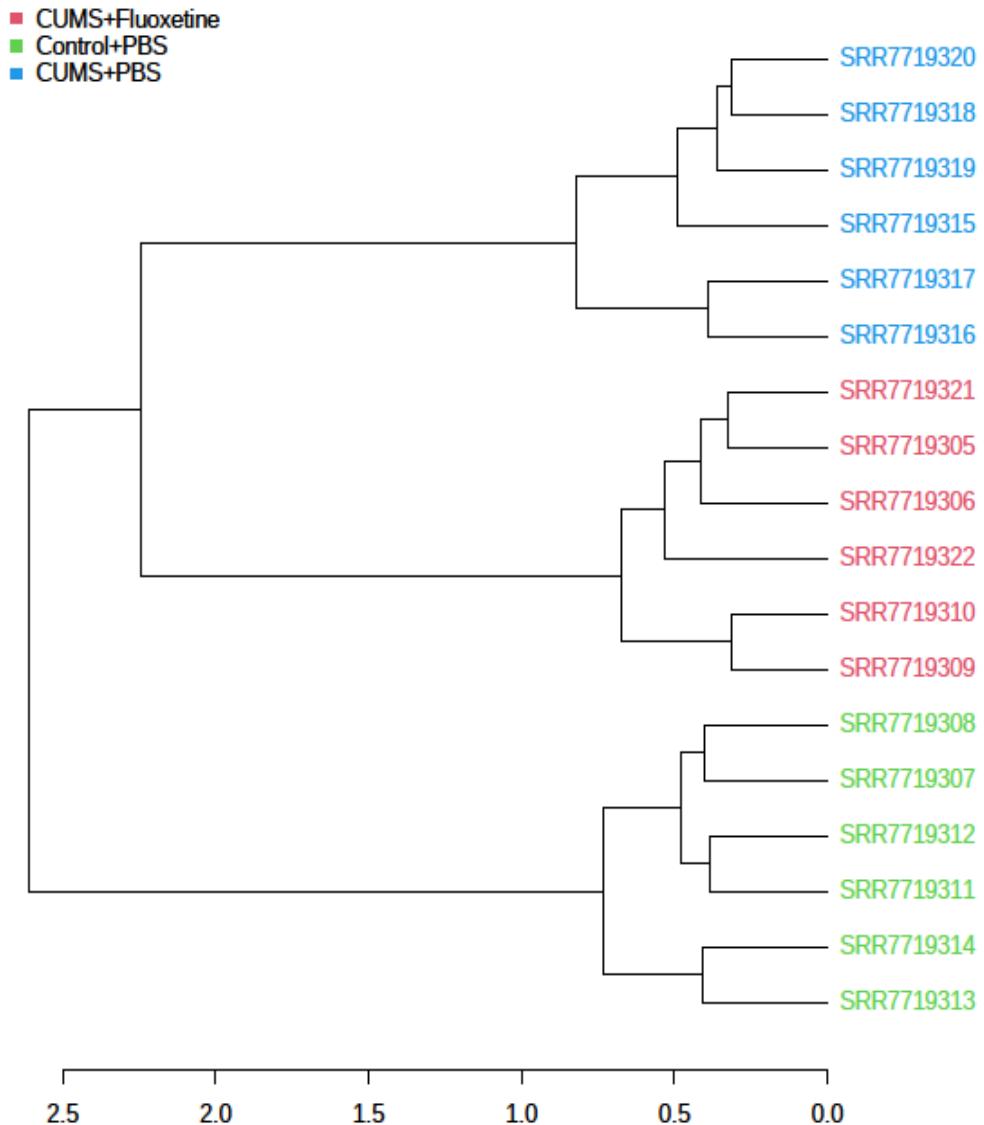
Supplementary Figure 1: Hierarchical dendrogram based on unweighted UniFrac distance. Unweighted Pair Group Method with Arithmetic mean revealed a noticeable distinction among groups (CUMS+Fluoxetine in red, Control+PBS in green, CUMS+PBS in blue), from the Sun 2019 study.
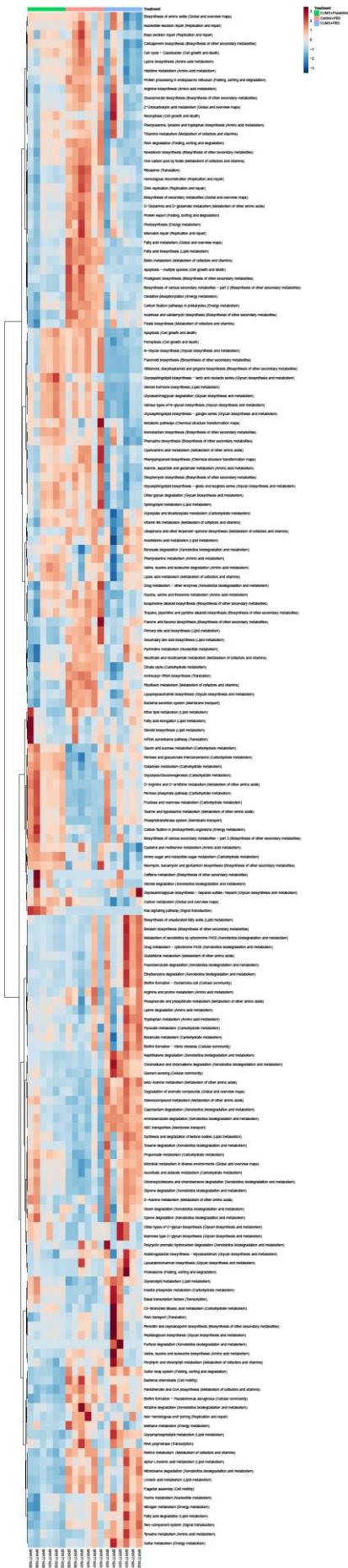
Supplementary Figure 2:
https://drive.google.com/file/d/1b-l89Z_oITD0sENaM87xvX6hdKqRmDhV/view?usp=sharing
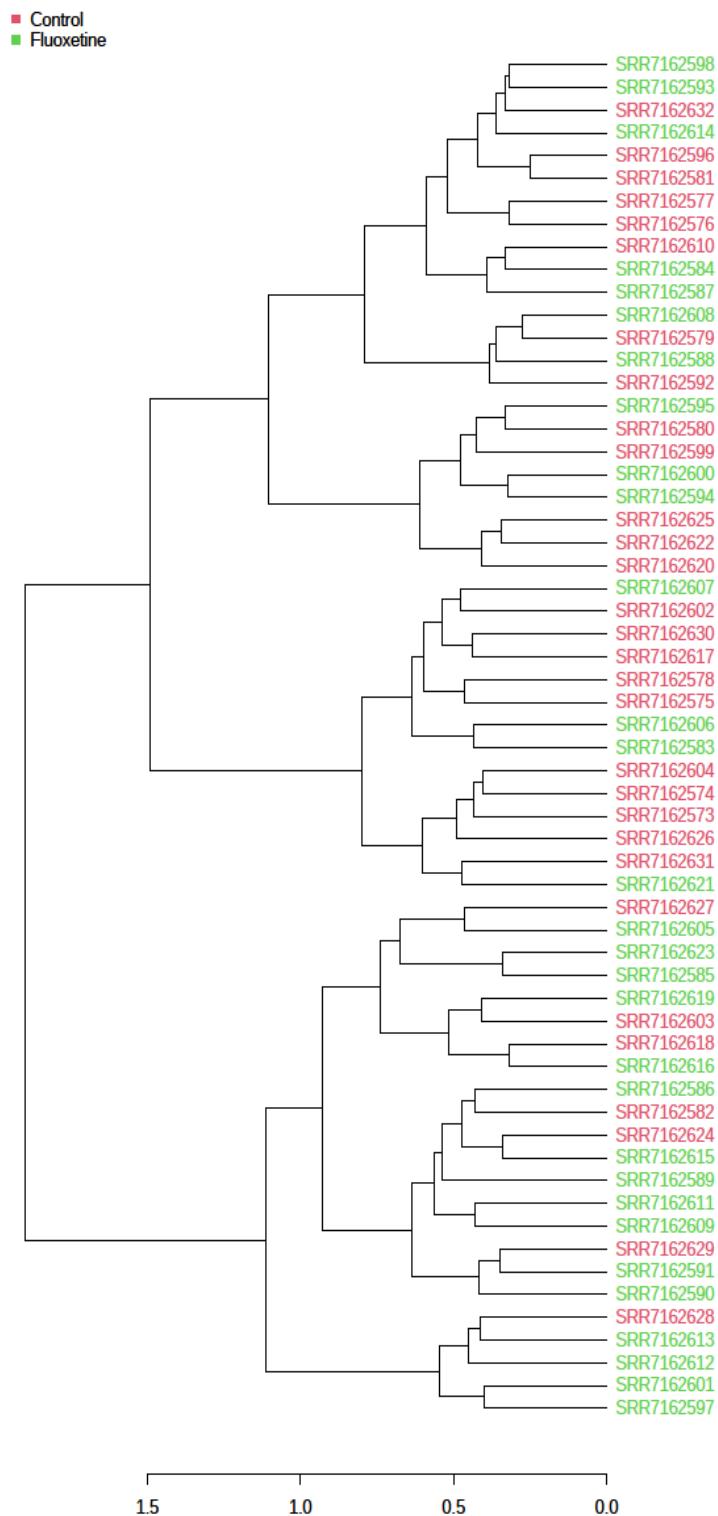
Supplementary Figure 3: Hierarchical dendrogram based on unweighted UniFrac distance. Unweighted Pair Group Method with Arithmetic mean clustering did not reveal a noticeable distinction among groups (Fluoxetine and Control), from the Lyte *et al.* (2019) study.

SUPPLEMENTARY FIGURES SUBTITLES

Supplementary Figure 1: Hierarchical dendrogram based on unweighted UniFrac distance. Unweighted Pair Group Method with Arithmetic mean revealed a noticeable distinction among groups (CUMS+Fluoxetine in red, Control+PBS in green, CUMS+PBS in blue), from the Sun 2019 study.

Supplementary Figure 2: Heatmap shows altered metabolic pathways among groups (CUMS+Fluoxetine in green, Control+PBS in pink, CUMS+PBS in blue)

Supplementary Figure 3: Hierarchical dendrogram based on unweighted UniFrac distance. Unweighted Pair Group Method with Arithmetic mean clustering did not reveal a noticeable distinction among groups (Fluoxetine and Control), from the Lyte 2019 study.

TABLES

Table 1: Papers selected through review of current literature.

	Article 1	Article 2
Title	Fluoxetine ameliorates dysbiosis in a depression model induced by chronic unpredicted mild stress in mice	Fluoxetine-induced alteration of murine gut microbial community structure: evidence for a microbial endocrinology-based mechanism of action responsible for fluoxetine-induced side effects
Authors	Sun L, Zhang H, Cao Y, Wang C, Zhao C, Wang H, Cui G, Wang M, Pan Y, Shi Y, Nie Y.	Lyte M, Daniels KM, Schmitz-Esser S.
Journal (Year)	Int J Med Sci (2019)	PeerJ. (2019)
Biosample code	PRJNA486701	SRP145610
Groups	Control (n=6) CUMS + PBS (n=6) CUMS + Fluoxetine (n=6)	Control (n=30) Fluoxetine (n=30)
Number of analyzed samples	18	60

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019).

'		P values	FDR	CUMS Fluoxetine	Contro PBS	CUMS PBS	LDAscore
Signal transduction)	Ras signaling pathway	31,453	11,557	13505	0	0	889
Amino acid metabolism	Glycine, serine and threonine metabolism	83,168	1,785	83248	85955	79163	3.53
	Alanine, aspartate and glutamate metabolism	68,172	16,096	95595	102420	85213	3.93
	Valine, leucine and isoleucine degradation	16,901	19,414	24720	22486	22139	3.11
	Valine, leucine and isoleucine biosynthesis	34,444	47,996	36504	40134	40025	3.26
	Tyrosine metabolism	44,745	12,678	13061	13513	17607	3.36
	Tryptophan metabolism	24,005	27,388	12670	12617	15415	3.15
	Phenylalanine, tyrosine and tryptophan biosynthesis	26,014	37,798	62015	68590	58997	3.68
	Phenylalanine metabolism	25,008	28,155	20929	20410	18784	3.03
	Lysine degradation	39,403	5,402	19788	16925	23592	3.52
	Lysine biosynthesis	13,124	23,485	49268	56715	45302	3.76
	Histidine metabolism	10,206	19,716	36499	40877	31075	3.69
	Cysteine and methionine metabolism	75,854	96,232	101420	99360	95188	3.49
	Arginine biosynthesis	18,316	30,246	45144	53505	46763	3.62
	Arginine and proline metabolism	3,855	41,742	44001	45084	45478	2.87
Biosynthesis of other secondary metabolites)	Biosynthesis of various secondary metabolites - part 2	14,644	11,557	5990.3	12866	6651	3.54
	Neomycin, kanamycin and gentamicin biosynthesis	95,142	19,028	7903.8	6496	5275.6	3.12
	Tropane, piperidine and pyridine alkaloid biosynthesis	87,152	1,785	8676.7	10321	7905	3.08
	Biosynthesis of various secondary metabolites - part 3	86,138	1,785	995.24	334.26	683.15	2.52
	Carbapenem biosynthesis	86,138	1,785	6951.6	7278.4	6180.5	2.74
	Flavone and flavonol biosynthesis	83,168	1,785	417.66	1516.8	850.8	2.74
	Penicillin and cephalosporin biosynthesis	69,996	72,118	3008.7	3383.2	4357.2	2.83

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019) (continuation).

		P values	FDR	CUMS Fluoxetine	Control PBS	CUMS PBS	LDAscore
Biosynthesis of other secondary metabolites	Phenazine biosynthesis	69,078	89,644	5530.9	5358.6	4321.7	2.78
	Monobactam biosynthesis	50,373	66,383	23910	23310	21528	3.08
	Prodigiosin biosynthesis	29,369	11,557	13792	20449	13309	3.55
	Flavonoid biosynthesis	27,539	11,557	1606	1970.3	484.76	2.87
	Stilbenoid, diarylheptanoid and gingerol biosynthesis	27,539	11,557	1606	1970.3	484.76	2.87
	Novobiocin biosynthesis	21,167	11,557	9980.3	12195	9144.9	3.18
	Acarbose and validamycin biosynthesis	15,986	11,557	7625.7	11959	9182.9	3.34
	Caffeine metabolism	13,708	16,527	3.9095	0	1.2221	471
	Betalain biosynthesis	11,142	20,814	119.56	166.59	560.07	2.34
	Streptomycin biosynthesis	7,708	17,471	36202	38405	31045	3.57
	Glucosinolate biosynthesis	2,468	37,237	6268.7	7532.7	6398.2	2.8
	Isoquinoline alkaloid biosynthesis	2,338	11,557	4120.6	5743.5	3659.6	3.02
Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	14,644	11,557	191380	166820	153260	4.28
	Pentose phosphate pathway	16,752	11,557	123710	75700	106230	4.38
	Fructose and mannose metabolism	18,941	11,557	179100	80064	144070	4.69
	Citrate cycle	21,167	11,557	63367	76307	56111	4
	Glycolysis/Gluconeogenesis	2,338	11,557	138050	111960	126910	4.12
	Pentose and glucuronate interconversions	30,956	11,557	77864	56099	69999	4.04
	Galactose metabolism	38,886	12,242	142050	100310	117000	4.32
	Ascorbate and aldarate metabolism	49,712	13,517	26763	16760	30783	3.85
	Propanoate metabolism	70,607	16,443	78189	64833	78123	3.82
	Starch and sucrose metabolism	74,859	17,197	141590	93552	132650	4.38

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019) (continuation).

'		P values	FDR	CUMS Fluoxetine	Control PBS	CUMS PBS	LDAscore
Carbohydrate metabolism	Inositol phosphate metabolism	12,378	22,386	17048	11812	17236	3.43
	Glyoxylate and dicarboxylate metabolism	2,411	37,237	78260	82824	73158	3.68
	C5-Branched dibasic acid metabolism	75,854	96,232	25339	23836	26659	3.15
	Pyruvate metabolism	1,197	14,821	112030	111850	116490	3.37
	Butanoate metabolism	33,112	36,317	62090	61654	64166	3.1
Cell growth and death	Apoptosis - multiple species	18,491	11,557	92779	849.78	16105	2.62
	Apoptosis	32,249	11,557	3316.2	3610	1068.4	3.1
	Ferroptosis	36,251	11,851	10321	12005	5753.6	3.5
	Cell cycle - Caulobacter	3,211	45,489	40267	42492	35491	3.54
	Necroptosis	5,805	60,543	14915	15007	14490	2.41
Cell motility	Bacterial chemotaxis	30,956	11,557	14150	36869	25953	4.06
	Flagellar assembly	14,752	2,559	16610	45349	40349	4.16
Cellular community	Quorum sensing	91,188	11,557	110910	96720	144610	4.38
	Biofilm formation - *Pseudomonas aeruginosa*	29,369	11,557	14361	22783	21192	3.62
	Biofilm formation - *Escherichia coli*	61,361	14,692	33256	34039	55697	4.05
	Biofilm formation - Vibrio cholerae	42,331	4,526	36218	37774	44146	3.6
Chemical structure	Metabolic pathways	95,142	19,028	2030100	2022800	2000600	4.17
	Phenylpropanoid biosynthesis	44,551	60,109	15924	21681	12017	3.68
Energy metabolism	Sulfur metabolism	94,445	11,557	36640	41638	50996	3.86
	Carbon fixation pathways in prokaryotes	25,523	11,557	83296	102790	78157	4.09
	Oxidative phosphorylation	33,992	11,557	92823	118090	92988	4.1
	Photosynthesis	50,889	13,517	24837	29007	23626	3.43

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019) (continuation).

		P values	FDR	CUMS Fluoxetine	Control PBS	CUMS PBS	LDAscore
Energy metabolism	Nitrogen metabolism	96,826	1,914	34592	38611	40432	3.47
	Carbon fixation in photosynthetic organisms	13,752	24,353	65502	56524	57652	3.65
	Methane metabolism	25,008	28,155	77300	81665	78639	3.34
Folding, sorting and degradation	RNA degradation	12,652	11,557	49861	57328	44395	3.81
	Protein export	21,167	11,557	50934	58815	48610	3.71
	Sulfur relay system	11,539	21,323	20486	27207	29711	3.66
	Protein processing in endoplasmic reticulum	1,668	28,355	6168.1	8096.6	5373.4	3.13
	Proteasome	23,922	37,237	7.7779	15111	74432	1.54
Global and overview maps	Microbial metabolism in diverse environments	2,338	11,557	528570	470620	545420	4.57
	Degradation of aromatic compounds	25,523	11,557	12193	10649	20073	3.67
	Fatty acid metabolism	33,206	11,557	46723	63145	47427	3.91
	Biosynthesis of secondary metabolites	33,401	11,557	834920	899920	817420	4.62
	Carbon metabolism	59,245	14,692	281490	279090	269790	3.77
	2-Oxocarboxylic acid metabolism	2,468	37,237	68532	77965	68894	3.67
	Biosynthesis of amino acids	28,111	31,235	380050	380690	354330	4.12
Glycan biosynthesis and metabolism	N-Glycan biosynthesis	2,338	11,557	1718.3	2083.1	612.41	2.87
	Glycosaminoglycan degradation	29,369	11,557	29474	27379	12417	3.93
	Glycosphingolipid biosynthesis - lacto and neolacto series	3,562	11,851	1175.4	566.29	198.74	2.69
	Glycosphingolipid biosynthesis - ganglio series	44,745	12,678	17371	16402	6613	3.73
	Various types of N-glycan biosynthesis	44,745	12,678	17400	16476	6629.9	3.73
	Lipopolysaccharide biosynthesis	50,889	13,517	35457	63504	38901	4.15
	Other glycan degradation	59,593	14,692	72547	71957	33164	4.29

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019) (continuation).

		P values	FDR	CUMS Fluoxetine	Control PBS	CUMS PBS	LDAscore
Glycan biosynthesis and metabolism	Glycosphingolipid biosynthesis - globo and isoglobo series	23,278	36,642	22858	23801	12791	3.74
	Lipoarabinomannan biosynthesis	40,272	5,477	19441	121.71	226.99	2.02
	Arabinogalactan biosynthesis - *Mycobacterium*	50,373	66,383	1415.1	1936.5	2027.5	2.49
	Mannose type O-glycan biosynthesis	16,384	18,947	94733	40214	108.49	1.55
	Other types of O-glycan biosynthesis	16,384	18,947	94733	40214	108.49	1.55
	Glycosaminoglycan biosynthesis - heparan sulfate / heparin	31,232	34,476	80156	39177	80253	1.33
	Peptidoglycan biosynthesis	73,779	75,557	65888	67294	70678	3.38
Lipid metabolism	Fatty acid biosynthesis	10,804	11,557	47218	63013	42829	4
	Fatty acid degradation	10,804	11,557	12174	15715	18746	3.52
	Secondary bile acid biosynthesis	1,685	11,557	646.87	3019.4	1555.5	3.07
	Glycerolipid metabolism	2,706	11,557	37752	27989	43131	3.88
	Primary bile acid biosynthesis	2,706	11,557	376.29	1653.8	823.71	2.81
	Glycerophospholipid metabolism	29,027	11,557	48952	54109	59188	3.71
	Linoleic acid metabolism	50,889	13,517	332.61	1623.9	1335.4	2.81
	Steroid hormone biosynthesis	61,361	14,692	7740.1	7487.4	3464	3.33
	Sphingolipid metabolism	83,168	1,785	43077	46665	22049	4.09
	Synthesis and degradation of ketone bodies	18,859	30,246	2068.3	1349.3	2994.4	2.92
	alpha-Linolenic acid metabolism	26,014	37,798	877.09	2094.3	2613.4	2.94
	Biosynthesis of unsaturated fatty acids	3,211	45,489	981.94	1124.8	2863.9	2.97
	Ether lipid metabolism	14,948	17,751	1646	2133.4	1607.5	2.42
	Fatty acid elongation	80,543	8,199	171.26	153.12	144.41	1.16
	Arachidonic acid metabolism	85,394	86,411	2877.1	3013.9	2554.5	2.36
	Steroid biosynthesis	97,688	97,688	155.28	122.67	95234	1.49

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019) (continuation).

		P values	FDR	CUMS Fluoxetine	Control PBS	CUMS PBS	LDAscore
Membrane transport	ABC transporters	10,804	11,557	230080	176910	339070	4.91
	Phosphotransferase system	32,249	11,557	211160	22251	179040	4.98
	Bacterial secretion system	51,789	13,545	49634	72573	61023	04.06
Metabolism of cofactors and vitamins	One carbon pool by folate	14,644	11,557	42570	50598	38081	3.8
	Pantothenate and CoA biosynthesis	16,752	11,557	48850	54238	52805	3.43
	Biotin metabolism	30,956	11,557	34822	50468	33871	3.92
	Folate biosynthesis	5,523	14,226	40485	59007	47361	3.97
	Riboflavin metabolism	10,206	19,716	21517	25257	22625	3.27
	Retinol metabolism	12,022	21,975	984.33	2790.9	2334.5	2.96
	Porphyrin and chlorophyll metabolism	38,718	53,512	48737	60035	62975	3.85
	Nicotinate and nicotinamide metabolism	51,565	67,431	43329	48610	46299	3.42
	Lipoic acid metabolism	78,105	98,354	7850.4	6505.9	5936.2	2.98
	Vitamin B6 metabolism	13,455	16,338	17928	19304	16607	3.13
	Ubiquinone and other terpenoid-quinone biosynthesis	15,036	17,751	25106	32020	27437	3.54
	Thiamine metabolism	16,035	18,799	41556	44170	39842	3.34
	Selenocompound metabolism	14,057	11,557	33789	30408	37102	3.52
	D-Arginine and D-ornithine metabolism	16,752	11,557	5010.7	256.5	2910	3.38
	beta-Alanine metabolism	43,202	12,678	12881	12304	16847	3.36
	D-Glutamine and D-glutamate metabolism	44,745	12,678	13014	15770	12993	3.14
	Taurine and hypotaurine metabolism	87,152	1,785	15924	12898	14738	3.18
	Cyanoamino acid metabolism	18,859	30,246	24684	31105	19220	3.77
	Glutathione metabolism	29,413	42,375	21931	21161	29789	3.63
	D-Alanine metabolism	13,455	16,338	9593.3	9031.4	10132	2.74

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019) (*continuation*).

		P values	FDR	CUMS Fluoxetine	Control PBS	CUMS PBS	LDAscore
Nucleotide metabolism	Pyrimidine metabolism	25,412	37,566	102650	109860	107390	3.56
	Purine metabolism	46,413	62,128	147660	152420	161560	3.84
Replication and repair	Homologous recombination	30,956	11,557	65232	79897	69287	3.87
	DNA replication	60,647	14,692	51676	59570	49523	3.7
	Non-homologous end-joining	86,138	1,785	25044	240.13	101.78	02.04
	Mismatch repair	24,971	37,237	61856	69380	62157	3.58
	Base excision repair	33,845	47,551	35677	36676	34584	03.02
	Nucleotide excision repair	10,341	12,927	31069	33040	29253	3.28
Signal transduction	Two-component system	43,202	12,678	211740	253520	276610	4.51
Transcription	Basal transcription factors	30,956	11,557	845.52	245.27	1235.1	2.7
	RNA polymerase	37,221	40,561	11758	12697	12451	2.67
Translation	Ribosome	27,539	11,557	197150	237350	199880	4.3
	Aminoacyl-tRNA biosynthesis	1,639	28,144	226770	299330	207900	4.66
	RNA transport	14,774	17,688	6104.2	6524.4	7125.1	2.71
	mRNA surveillance pathway	5,225	55,171	120.26	122.67	63465	1.49
Xenobiotics biodegradation and metabolism	Caprolactam degradation	12,652	11,557	1435.6	1021.3	3010.4	3
	Chloroalkane and chloroalkene degradation	16,752	11,557	3215.5	2527.8	9549.9	3.55
	Aminobenzoate degradation	18,941	11,557	7117.7	5267.7	11519	3.5
	Naphthalene degradation	29,369	11,557	1860	1836.1	6257.7	3.34
	Chlorocyclohexane and chlorobenzene degradation	38,886	12,242	2854	529.28	3561.1	3.18
	Styrene degradation	10,632	20,083	3237.2	1288.4	4144.7	3.16
	Nitrotoluene degradation	10,632	20,083	2414.3	6526.3	6835.9	3.34
	Xylene degradation	14,243	24,963	5019.5	2421.3	4710.5	3.11

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun *et al.* (2019) (*continuation*).

		P values	FDR	CUMS Fluoxetine	Control PBS	CUMS PBS	LDAscore
Xenobiotics biodegradation and metabolism	Ethylbenzene degradation	17,997	30,246	601.26	609.95	1752.2	2.76
	Toluene degradation	18,859	30,246	986.01	482.87	1679.9	2.78
	Drug metabolism - cytochrome P450	18,859	30,246	1003.8	1329.4	3861.2	3.16
	Fluorobenzoate degradation	23,278	36,642	245.35	387.71	1279.2	2.71
	Metabolism of xenobiotics by cytochrome P450	24,971	37,237	1082.1	1365.4	3937.7	3.15
	Atrazine degradation	70,713	9,107	721.39	1969.6	1177.7	2.8
	Furfural degradation	12,031	14,821	0	0	2.9511	394
	Drug metabolism - other enzymes	28,111	31,235	31482	33857	31567	03.07
	Dioxin degradation	39,927	42,959	2630.4	2264.5	2907	2.51
	Benzoate degradation	45,943	48,814	17322	16315	17609	2.81
	Steroid degradation	52,865	55,476	112.83	44829	76455	1.54
	Polycyclic aromatic hydrocarbon degradation	92,531	93,079	13739	27.16	25467	887

Table 3: LEfSe analysis of distinct pathways from metabolic prediction, from Lyte *et al.* (2019).

		P values	FDR	Control	Fluoxetine	LDAscore
Biosynthesis of other secondary metabolites	Penicillin and cephalosporin biosynthesis	0.011959	0.28768	7415.2	6603.0	-2.61
	Glucosinolate biosynthesis	0.014119	0.28768	6833.0	7463.7	2.5
Carbohydrate metabolismo	Ascorbate and aldarate metabolism	0.038469	0.44789	9569.2	10981.0	2.85
Cell growth and death	Necroptosis	0.047579	0.44982	15838.0	16809.0	2.69
Cellular Community	Biofilm formation - *Escherichia coli*	0.014119	0.28768	20435.0	22028.0	2.9
Folding, sorting and degradation	RNA degradation	0.029757	0.38727	66942.0	65638.0	-2.81
Global and overview maps	Microbial metabolism in diverse environments	0.0047454	0.25783	443690.0	451760.0	3.61
	Biosynthesis of amino acids	0.013549	0.28768	374860.0	387550.0	3.8
Lipid metabolism	Glycerophospholipid metabolism	0.012999	0.28768	61751.0	59370.0	-3.08
Metabolism of cofactors and vitamins	Nicotinate and nicotinamide metabolism	0.0041284	0.25783	52310.0	51182.0	-2.75
	Ubiquinone and other terpenoid-quinone biosynthesis	0.049261	0.44982	23047.0	21659.0	-2.84
Metabolism of other amino acids	D-Glutamine and D-glutamate metabolism	0.016617	0.30095	18834.0	17999.0	-2.62
Nucleotide metabolismo	Pyrimidine metabolism	0.030887	0.38727	128730.0	123590.0	-3.41
Replication and repair	Base excision repair	0.024625	0.3649	46868.0	44694.0	-3.04
Translation	RNA transport	0.0032599	0.25783	9033.4	8573.7	-2.36
Translation	Aminoacyl-tRNA biosynthesis	0.045945	0.44982	417560.0	400310.0	-3.94
Xenobiotics biodegradation and metabolism	Drug metabolism - other enzymes	0.020278	0.33054	39132.0	37907.0	-2.79

TABLES SUBTITLES

Table 1: Papers selected through review of current literature.

Table 2: LEfSe analysis of distinct pathways from metabolic prediction, from Sun, 2019 study.

Table 3: LEfSe analysis of distinct pathways from metabolic prediction, from Lyte, 2019 study.

4. CONSIDERAÇÕES FINAIS

As pesquisas com potenciais marcadores de predição para doenças psiquiátricas têm sido um tema recorrente. O estresse se mostrou um importante fator de impacto no desequilíbrio de populações bacterianas intestinais, que por sua vez afetam a saúde mental, um ciclo com potencial de se retroalimentar de maneira crônica se não for interrompido.

Embora mais antiga, a fluoxetina ainda é uma medicação barata e efetiva na redução da morbidade em doenças nas quais o estresse tem um papel preponderante, como depressão e transtornos de ansiedade. Entender melhor por quais vias o estresse pode modular a disbiose, e qual o papel da fluoxetina no controle da mesma é essencial para o desenvolvimento de novos fármacos com foco na modulação de rotas metabólicas ou da própria microbiota de forma a restituir a saúde mental de forma mais rápida e eficaz.

Este estudo foi possível graças à disponibilização pública e gratuita dos dados coletados. Com o advento de novas técnicas de sequenciamento de alto rendimento, permitindo a análise de bases de dados gênicas cada vez maiores, de forma mais eficiente e barata, abre-se também a possibilidade do compartilhamento mais amplo de dados científicos, com intuito de aumentar o tamanho amostral e acurácia dos métodos analíticos.