Investigation of Stress Situation of Pediatric Nurses and Corresponding Changes of Intestinal Flora

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Abstract

BACKGROUND: Sequencing the stool samples to explore the relationship between intestinal flora and the stressor of nurses.

METHODS: The research take the pediatric nurses in the Third Xiangya Hospital as the research group and the internal medicine nurses as the control group. Use the "Chinese Nurse Stressor Scale"(CNSS) for general investigation and stress-related assessment. 6 in internal medicine group and 7 in pediatric group to conduct cell sequencing of intestinal flora. Gut microbiome was profiled by 16S rRNA sequencing.

RESULTS: The work stressors of internal medicine and pediatric nurses includes nursing specialty and work (10.94±3.17 vs 12.25±2.78), work environment and resources (4.91±1.63 vs 5.66±2.03), patient care (15.86±4.30 vs 18.95±5.12) and management and interpersonal relationship (8.83±4.94 vs 10.86±5.01). The results were statistically different ($P < 0.05$). The abundance of *Clostridia* in internal medicine group is higher, while the abundance of *Bacteroidia* in pediatric group is higher. The abundance of *γ-proteobacteria* and *β-proteobacteria* also have obvious differences between two groups.

CONCLUSIONS: The overall stress score of the pediatric nurse group was higher, with their stool samples having more abundance of *Bacteroidia,* but *Clostridia, γ-Proteobacteria* and *β-Proteobacteria* are fewer than that in internal medicine nurses.

1 Background

With the enforcing of the second-child policy since 2015 in China, the number of infants and children has been increasing, and the overall social attention to the health of children has increased significantly[1]. However, the number of pediatric nurses in China is relatively small, resulting in the workload of nurses far exceeding the normal standard. This will be accompanied by increased stress concerning career promotion, working quality, work enthusiasm, doctor-patient communication or other aspects[2]. There are also stressors for pediatric nurses especially in the aspects such as the misunderstanding from anxious parents, the specificity of work in pediatrics and career promotion pressure which decreases the sense of security of nurses[3, 4]. Meanwhile, nurses who prepared for pregnancy usually face more psychological pressure than ordinary pregnant women[5]. Under such pressure, the mental health status of nurses is generally at a terrible stage. Some may even leave the job, indirectly leading to the insufficient staffs and overloaded work among pediatric nurses.

In recent years, the relationship between intestinal flora and central nervous system diseases has become a research hotspot[6, 7]. Intestinal microorganisms (bacteria, fungi, viruses, etc.) are a total collection of billions of microorganisms residing in the human gastrointestinal tract, of which bacteria contains about 1,000 different species [6, 8]. Metagenomic analysis of gastrointestinal bacteria has shown that it contains more than 3 million unique genes, more than 100 times the number of human genes, and formed a very diverse ecosystem[9–11]. Intestinal bacteria enable the growth of metabolically active flora by assisting in the digestion of indigestible compounds (dietary fiber, etc) and maximizing the provision and bioavailability of essential nutrients[2, 12]. If intestinal mucosal barrier cells are destroyed, however, a large number of opportunistic pathogens, such as *Escherichia coli* and *enterococcus faecalis*, will disrupt the ecological balance of microbial community[6, 13]. The brain-gut axis is associated with the pathogenesis of mental illness, with various roles such as regulating immunity, promoting digestion and metabolism, affecting the absorption of fat[14], and more importantly, an connection between intestinal flora and stress. Studies have suggested that the intestinal flora and brain-gut axis connect the central nervous system (CNS) with the enteric nervous system (ENS) and neuro-endocrine-immune system to form a two-way communication pathway, which regulates gastrointestinal motility function, visceral sensitivity, brain-gut peptide secretion, body responsiveness to stress, and central cognitive function[15]. So far, there are

few studies on intestinal flora and occupation-related stress, especially among pediatric nurses, so this is a topic worthy of attention.

In this experiment, for one thing, pediatric nurses were given questionnaires and their stress state was evaluated by scales. For another, their gut flora from stool samples was sequenced to seek for differences. This study aims to pay attention to the physical and mental health of pediatric nurses and analyze the current status of stressors in pediatric nurses. In this way, some corresponding measures can be proposed to improve the working atmosphere of pediatric nurses.

2 Methods

2.1 Participants

In this study, we used online survey questionnaires and anonymous submission of questionnaires to conduct pressure-related surveys on pediatric and internal medicine nurses at The Third Xiangya Hospital of Central South University. All participants meet the following criteria: (1) registered nurses registered in The Third Xiangya Hospital;(2) female in good health, not pregnant or lactating; (3) known the project process of this study and been willing to cooperate with the investigation; (4) exclude those who have severe mental illness or have been diagnosed as anxiety disorder or depression anxiety; (5) no related gastrointestinal diseases or recent gastrointestinal symptoms; (6) there were no major family changes or other events that seriously affected the mood of the test subjects recently; (7) the objects have passed the relevant ethical review. (8) exclude who have history of drugs, antibiotics, probiotics or prebiotics taken within 3 months. This study was reviewed and approved by the hospital ethics committee, and informed consent was signed by all nurses investigated.

2.2 Research content

2.2.1 Questionnaire content

Chinese nurses' stressors scale was selected for stress-related investigation. This scale investigates 35 questions from five aspects: nursing specialty and work problems, workload and time allocation problems, work environment and resources problems, patient care problems and management and interpersonal relationship problems. Each question is divided into four grades: 0, 1, 2 and 3. The higher the score of the answer is, the more pressure the nurse has. Therefore, the questionnaire we selected is scientific, universal and comprehensive in content coverage. (Supplement1)

2.2.2 Experimental content

In order to ensure the randomness of the sample, we selected 35 and 44 nurses by random number table respectively. Fresh feces with 7 internal medicine nurses(in control group) and 6 pediatric nurses(in experimental group) were picked out for sequencing. The sampler intercepts the middle part of the sample, distributes it to a sterilized cryopreservation tube, and stored in a -80 ℃ refrigerator for future use. Total DNA extraction was performed using a QIAamp® DNA Stool Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. The 16S rDNA sequencing selection region is V1-V2 variable region, and the universal primers used are 27F (5'AGAGTTTGATCCTGGCTCAG-3') and 338R (5'-TGCTGCCTCCCGTAGGAGT-3'). Products amplified by PCR were purified and quantified using the Qubit dsDNA BR Assay Kit.

OTU (operational taxonomic units) is a unified mark set for a taxon (strain, genus, species, group, etc.) in the study of phylogeny or population genetics for the convenience of analysis. In order to understand the number of species, genera and other information in the sequencing results of a sample, we need to cluster the sequence. Through the classification operation, the sequences are classified into many groups according to their similarity, each group is an OTU. In general,

tags with similarity of more than 97% are clustered into an OTU. Using software usearch(v7.0.1090). Cluster the spliced tags into OTU. The main process is as follows: 1) using uparse to cluster under 97% similarity to get the representative sequence of OTU; 2) using uchime(v4.2.40). The mosaics produced by PCR were removed from the OTU representative sequences (16S compared with the existing mosaics database to remove the mosaics. 18S adopts denovo method to remove the chimera 16S chimera database: golddatabase (v20110519) its chimera database: unite (v201407 03), divided into its full length, ITS1 and ITS2, selected according to sequencing area); 3) using usearch–the global method compares all tags back to the OTU representative sequence, and obtains the OTU abundance statistical table of each sample.

PLS-DA analysis (PLS-DA) is a multivariate statistical analysis method for discriminant analysis, which is often used to determine how to classify the research objects. Compared with PCA, PLS-DA method can combine the function of principal component analysis and multiple regression to maximize the interval between components of PCA. That is to say, compared with PCA analysis, the interval between groups in PLS-DA analysis results is larger, and it is easier to see the differences between groups.

2.3 Statistical analysis

The subjects filled out questionnaires without giving their names. We summarized the results, entered the data in Excel, and conducted statistical analysis with SPSS 22.0. Quantitative variables are expressed as mean values with standard deviations. Quantitative variables are expressed in Numbers and percentages. T test was used to compare the differences in the total stress scores and the scores of each dimension among nurses in different departments. *P* value less than or equal to 0.05 was considered statistically significant.

3 Results

3.1 Stressors questionnaire results

A total of 35 internal medicine nurses and 44 pediatric nurses participated in the trial. Their basic information and corresponding proportions are described in detail in Table 1. There was a significant difference in the number of years of service between the control group and the experimental group (66% of nurses with more than 10 years of service in internal medicine versus 32% in pediatrics). In addition, the proportion of medical nurses over 30 years old (71%) was higher than that of pediatrics (57%). The proportion of people with educational background and marriage status is relatively consistent.

The scores of internal medicine nurses and pediatric nurses in various dimensions of work stressors are shown in Table 2. In terms of nursing specialty and work (10.94 ± 3.17 vs 12.25 ± 2.78), work environment and resources (4.91 ± 1.63 vs 5.66 ± 2.03), patient care (15.86 ± 4.30 vs 18.95 ± 5.12) and management and interpersonal relationship (8.83 ± 4.94 vs 10.86 ± 5.01), there were statistically significant differences between the control group and the experimental group. Meanwhile, the overall score of internal medicine nurses is 50.17 ± 12.13 while pediatric nurses is 57.93 ± 13.01($P < 0.05$).

The results of the objective factors of nurses' stressors in different departments were further compared, as shown in Table 3. The scores of pediatric nurses who worked for more than 10 years (63.50 ± 13.06) were higher than those of internal medicine nurses (47.96 ± 11.98). The pressure of pediatric nurses over 30 years old (59.56 ± 12.92) was also higher than that of medical nurses (48.72 ± 12.49). The stress score of the highly educated pediatric nurses (59.40 ± 14.20) was higher than that of the internal medicine nurses (45.00 ± 10.65). In addition, the stress of married pediatric nurses (59.18 ± 14.12) was higher than that of internal medicine nurses (50.68 ± 12.81). The results are statistically different($P < 0.05$).

3.2 Intestinal flora analysis results

6 nurses selected randomly from internal medicine were set as the control group (group A) while 7 from pediatric were the experimental group (group B) for further analysis by 16S amplification. The structure and abundance of intestinal flora between the two groups showed significant differences. Partial least squares discrimination analysis (PLS-DA) was used to explore the differences in the structure of intestinal flora between groups A and B. The result can be seen in Fig. 1.

Species abundance heat map is a graphical display method of clustering according to the similarity of species or sample abundance by the color gradient representing the relative abundance of species. It can intuitively display the composition and proportion of each sample species, reflecting the changes of species between samples. As can be seen from the figure, the main difference between the two groups exists in *Clostridia* and *Bacteroidia*. The abundance of *Clostridia* in group A is higher than that in group B, whereas *Bacteroidia* is lower than that in group B. There are also differences in abundance of *γ-Proteobacteria* and *β-Proteobacteria* between the two groups(Figs. 2 and 3).

4 Discussion

It can be clearly seen from the survey results that the overall pressure of pediatric nurses is relatively high higher than internal nurses. It is mainly reflected in nursing specialty, working environment and resources, patient care, management and interpersonal relationship. Working experience, marriage condition and educational background are all factors influencing the stress levels of pediatric nurses. Lupien et al. have shown that nurses experience high levels of test anxiety, state anxiety, and stress, which can be improved after intervention[16]. Sonia et al. point out that the effects of stress on the brain and behavior in adulthood are reversible and usually disappear when the stressor stops[17]. Thus, analysis of possible stressors is important to deal with the pressure of pediatric nurse.

One of the critical reasons is the particularity of the pediatric nurses. Children have weak immune capacity and insufficient regulatory adaptability because of immature development and imperfect system function[18]. The disease has the characteristics of rapid onset and rapid development of the disease process and the prognosis is not optimistic, which cause pediatric nurses to have heavy work content, significant responsibility, and greater pressure than internal medicine. Secondly, the second child policy in China in recent years generate a fertility boom, but the number of pediatric staff and the overall scale of pediatrics have not been expanded enough, which has increased the workload of pediatric medical staff. The increasing number of pediatric patients lead to the insufficiency of hospital capacity. Long waiting time for medical consultation and impatience among parents can easily spur conflicts between nurses and patients, or even escalates to the workplace violence[19]. Studies have shown that emergency and pediatrics are the two most vulnerable departments for nurses to meet violence at work[20]. Thirdly, in aspects of working environment, pediatric nurses have a low status, low rewards, more onerous work and more complex doctor-patient relationship compared with other departments[19], which aggravates the loss of personnel of pediatric nurses and makes it more difficult for pediatrics to recruit medical staff. Many hospitals have experienced pediatric "employment shortage". The lack of nursing staff, the heavy workload of nurses and the harsh working environment increase the subjective anxiety and pressure of nurses, leading to the stress among pediatric nurses generally higher than that among internal medicine nurses. A lot more reasons are worthy to consider, and our experiment looks at the problem from a novel perspective.

By comparing stool samples from pediatric nurses and internal medicine nurses, we found significant differences in the structure and abundance of intestinal bacteria (Fig. 1). Pediatric nurses had lower abundance of *H.ammodendron* but higher *Bacteroides* than medical nurses. Meanwhile, the abundances of *γ-Proteobacteria* and *β-Proteobacteria* were also varied in the bunch (Fig. 2, Fig. 3). Consistent with this, the analysis of the stress scores did show that the total stress

factor scores and four dimensions of pediatric nurses were considerably higher than that of internal medicine nurses, and the differences were statistically significant.

This interesting consistency suggests a role for the brain's gut axis. The stress of pediatric nurses is closely related to the psychological effects of work stress. The external stimulation generated by working pressure influences intestinal activities through direct and indirect ways, causing changes in the release of duodenal bicarbonate, hormonal changes, and decreased mucosal immune function[21], and even intestinal diseases such as intestinal stress syndrome may occur. Enteropathy affects the central nervous system through changes in brain-gut peptide secretion. Anxiety, depression and other emotions of pediatric nurses continue to intensify, forming a vicious circle, and eventually lead to differences in the composition of intestinal bacteria between pediatric nurses and medical staff.

Existing studies have shown that biofeedback therapy and behavioral relaxation therapy can improve these symptoms[22]. Bravo et al. found that probiotics can regulate GABA receptor expression in the cerebral cortex through the vagus nerve, thereby reducing anxiety and depressive behaviors[23]. Sharon et al. demonstrated that serum 4-ethylphenyl sulfate (4EPS) concentrations were increased in autistic mice, and after treatment with B. fragilis, 4EPS concentrations were decreased and anxiety behaviors were alleviated in mice[24]. These studies suggest that due to the characteristics of bidirectional communication of the brain-gut axis, it can also act on the central nervous system through the regulation of intestinal bacteria, providing a new therapeutic idea for clinical practice. At the same time, the latest research shows that non-probiotic interventions such as resistant dextrin supplementation can achieve better results, so the effect of dietary restructuring is better than probiotic supplements[25].

Numerous studies have shown that psycho-psychological factors are closely linked to intestinal motility disorders and visceral sensitivity mechanisms[26]. Behavior and cognition can affect intestinal activity through indirect complex pathways. After feeling external stimuli, the action pathways of the brain-gut axis on the intestine include indirect pathways and direct pathways. The indirect pathway is through changes in gastrointestinal motility and secretion or changes in intestinal permeability. Direct pathways include the release of signaling molecules into the luminal lamina propria of intestinal cells (cells of enterochromaffin cells, neural cells, immune cells). Changes in intestinal activity, on the other hand, can affect changes in intestinal bacteria. For example, de Magistris's et al. found that autistic patients had increased intestinal mucosal permeability and increased blood concentrations of bacterial lipopolysaccharide (LPS), indicating that brain-gut axis dysfunction can disrupt the intestinal mucosal barrier causing bacterial translocation[27]. In addition, the brain-gut axis causes irritable bowel syndrome (IBS) under stress conditions. IBS is a common functional bowel disorder which is closely related to the function of brain-gut axis. Since the bidirectional pathway of brain-gut axis also acts on the central nervous system, the generation of IBS will also have adverse effects on the patient's mood. Brilliant et al., after acupuncture and oral treatment of trimebutine maleate tablets in IBS patients, found that while IBS symptoms were relieved, brain-gut peptide secretion such as 5-HT, NPY, and CCRP was reduced, and mental stress was significantly reduced[28].

As a result, appropriate use of psychological biological agents, antibiotics and other drugs to regulate intestinal flora, through the intestinal and brain axis action to alleviate relatively serious psychological stress (such as anxiety, depression, etc.)[9, 29, 30]. Probiotics and prebiotics can be considered taken in daily work for pediatric nurses so as to moderately supply for reduced intestinal flora. From the perspective of traditional Chinese medicine, proper diet therapy can also improve the type and quantity of intestinal flora.

True, merely finding for a curable treatment to reduce psychological stress is a limited approach, which could only treat the symptoms but not the root causes. In view of this situation, pediatric nurses requires an appropriate policy and a better working environment to relieve potential stress. Hospitals should appropriately expand the scale of pediatrics to adapt to the “second-child policy” to ensure the workload of pediatric nurses within acceptable limits. Adopt more diversified management strategies, such as “double night shifts”. Under this policy, both physical need for rest and work

effectiveness can be accommodated among the pediatric nurses. Managers could care about nurses such as organizing mindful exchange meetings to eliminate the impact of work stress. For the nurses themselves, improving their professionalism, correctly balancing the relationship between family and work and finding suitable channels for stress relief are also effective ways to relieve stress.

5 Conclusion

The psychological stress of pediatric nurses is generally more serious than that of internal medicine nurses due to the surging number of children, large workload, poor working environment and the tension between pediatricians and patients. Considering that intestinal flora and psychological stress may interact through the brain-gut axis, we tested intestinal flora samples from people with high stress. Further analysis of the types and quantities of intestinal flora found that pediatric nurses had more *Bacteroidia*, but fewer *Clostridia, γ-Proteobacteria* and *β-Proteobacteria* than medical nurses. More relevant clinical intervention studies can be carried out to elucidate the relationship between intestinal bacteria regulation and pressure improvement by focusing on the regulation of intestinal bacteria through the non-probiotic pathway in the future.

Abbreviations

All abbreviations are defined in the manuscript.

Declarations

Ethics approval and consent to participate:

This study was approved by the institutional review board of the Third Xiangya Hospital Central South University (Permission Number: 20020).

Consent for publication:

Not applicable.

Availability of data and material:

The data used to support the findings of this study are included within the article.

Competing interests:

The authors declare no conflict of interest, financial or otherwise.

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Authors' contributions:

ZW and BJ conceived of and designed the study and drafted the manuscript. JH and QW performed the literature search and performed the comparison. RS conduct data analysis of questionnaire results. ZC and ZZ generated the figures and tables and performed the background research. JH and JS edited the manuscript. XZ revised the manuscript for

important intellectual content. MZ and XZ are responsible for the full text. All authors have read and approved the content of the manuscript.

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Tables

Table 1

Baseline data of participants

Department	total	Age		Working years		Educational background		Marital status	
		< 30	≥ 30	≤ 10	> 10	Bachelor	Master	Unmarried	Married
Internal medicine	35	10(0.29)	25(0.71)	12(0.34)	23(0.66)	26(0.74)	9(0.26)	7(0.20)	28(0.80)
Pediatric	44	19(0.43)	25(0.57)	30(0.68)	14(0.32)	34(0.77)	10(0.23)	11(0.25)	33(0.75)

Table 2

The score of overall and each dimension

Department	Number	Nursing specialty and work	Workload and time allocation	Work environment and resources	Patient care	Management and interpersonal relationship	Total score
Internal medicine	35	10.94 ± 3.17	9.63 ± 3.18	4.91 ± 1.63	15.86 ± 4.30	8.83 ± 4.94	50.17 ± 12.13
Pediatric	44	12.25 ± 2.78	10.20 ± 2.68	5.66 ± 2.03	18.95 ± 5.12	10.86 ± 5.01	57.93 ± 13.01
P		< 0.05	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 3

Comparison of objective factors of stressors

Department		Internal medicine	Pediatric	P
Working years	≤ 10	54.42 ± 11.74	54.75 ± 12.08	> 0.05
	> 10	47.96 ± 11.98	63.50 ± 13.06	< 0.05
Age/ years old	< 30	53.80 ± 10.92	55.79 ± 13.16	> 0.05
	≥ 30	48.72 ± 12.49	59.56 ± 12.92	< 0.05
Educational background	Bachelor's degree	51.65 ± 12.53	56.53 ± 12.34	> 0.05
	Master's degree or above	45.00 ± 10.65	59.40 ± 14.20	< 0.05
Marital status	Unmarried	48.14 ± 9.44	54.18 ± 8.32	> 0.05
	Married	50.68 ± 12.81	59.18 ± 14.12	< 0.05

Figures

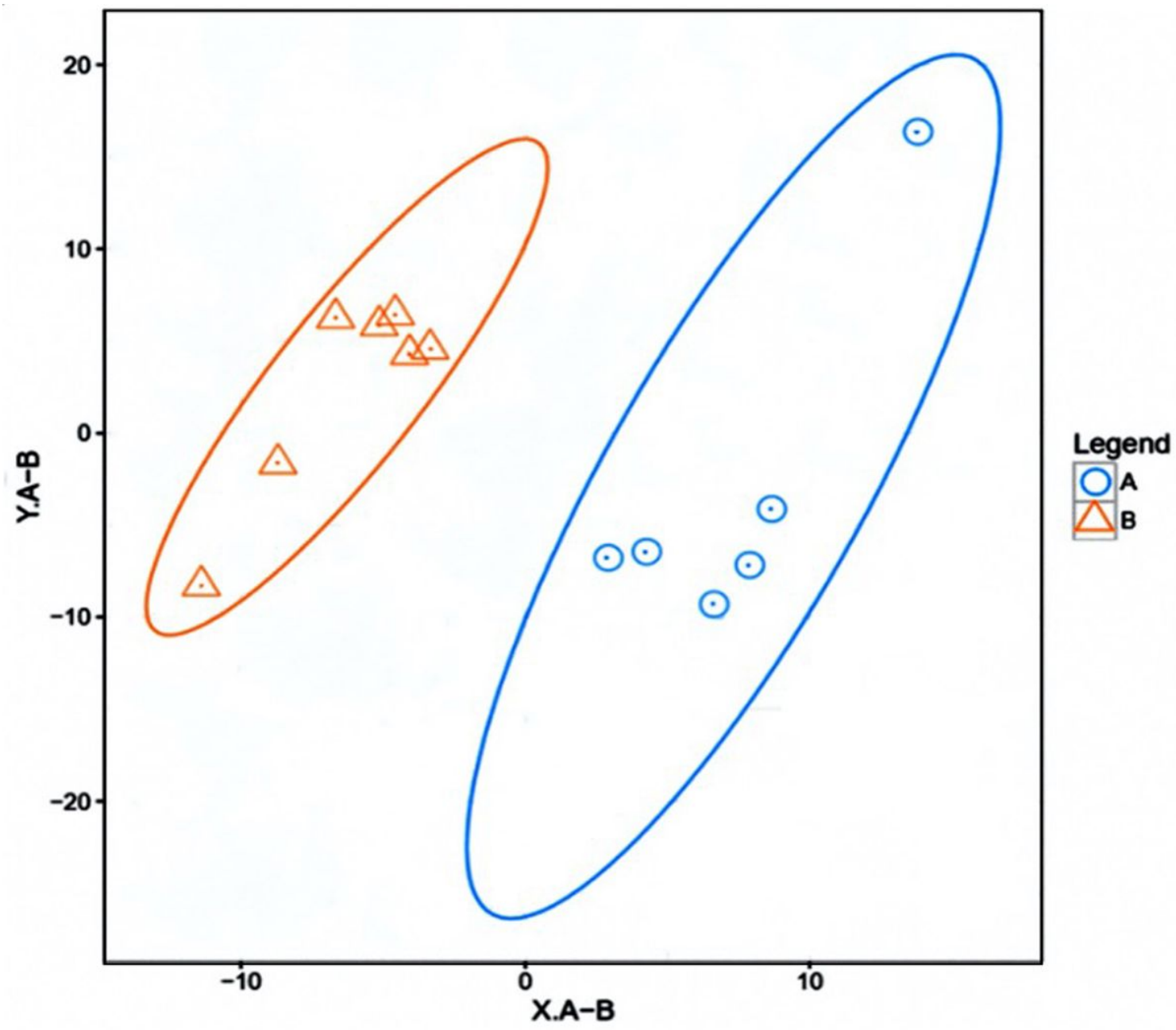

Figure 1

OTU based PLS-DA analysis *PLS-DA is a multivariate statistical analysis method used for discriminant analysis. It can be seen from the figure that the main difference between the two groups A / B exists in Clostridia and Bacteroidia, which have a high proportion. (A: control group, internal medicine nurses; B: experimental group, pediatric nurses)

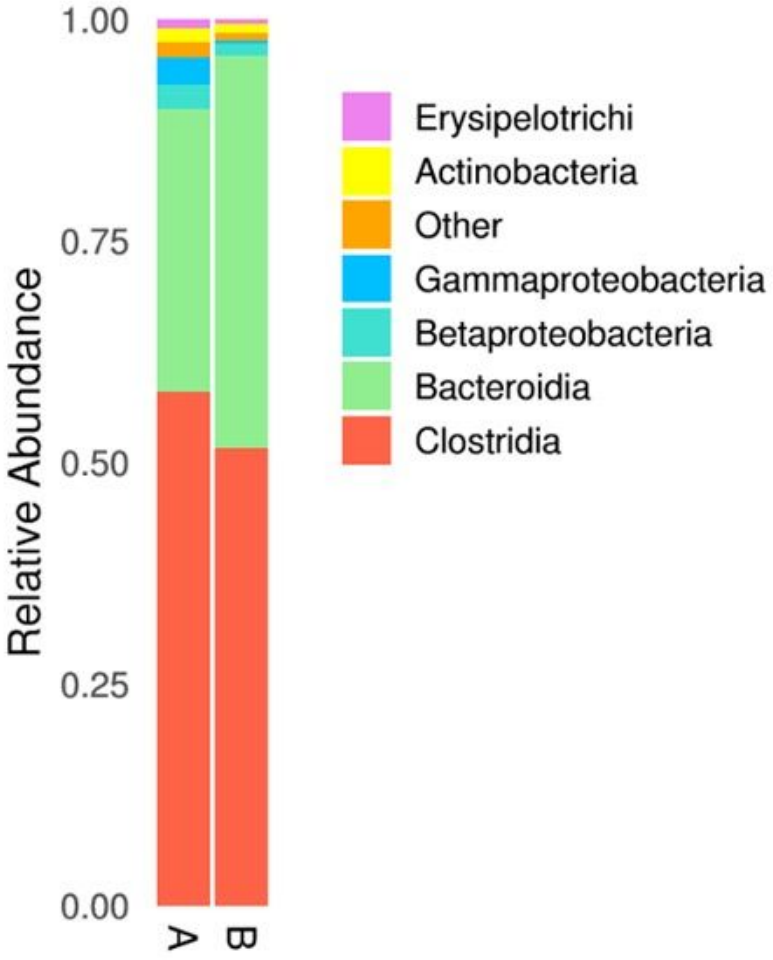

Figure 2

The species abundance histogram about relative abundance of different flora. *Species abundance histogram can intuitively display the composition and proportion of each sample species, reflecting the changes of species between samples. The abundance of Clostridia in group A is higher than that in group B, and the abundance of Bacteroidia in group B is higher than that in group A. (A: control group, internal medicine nurses; B: experimental group, pediatric nurses)

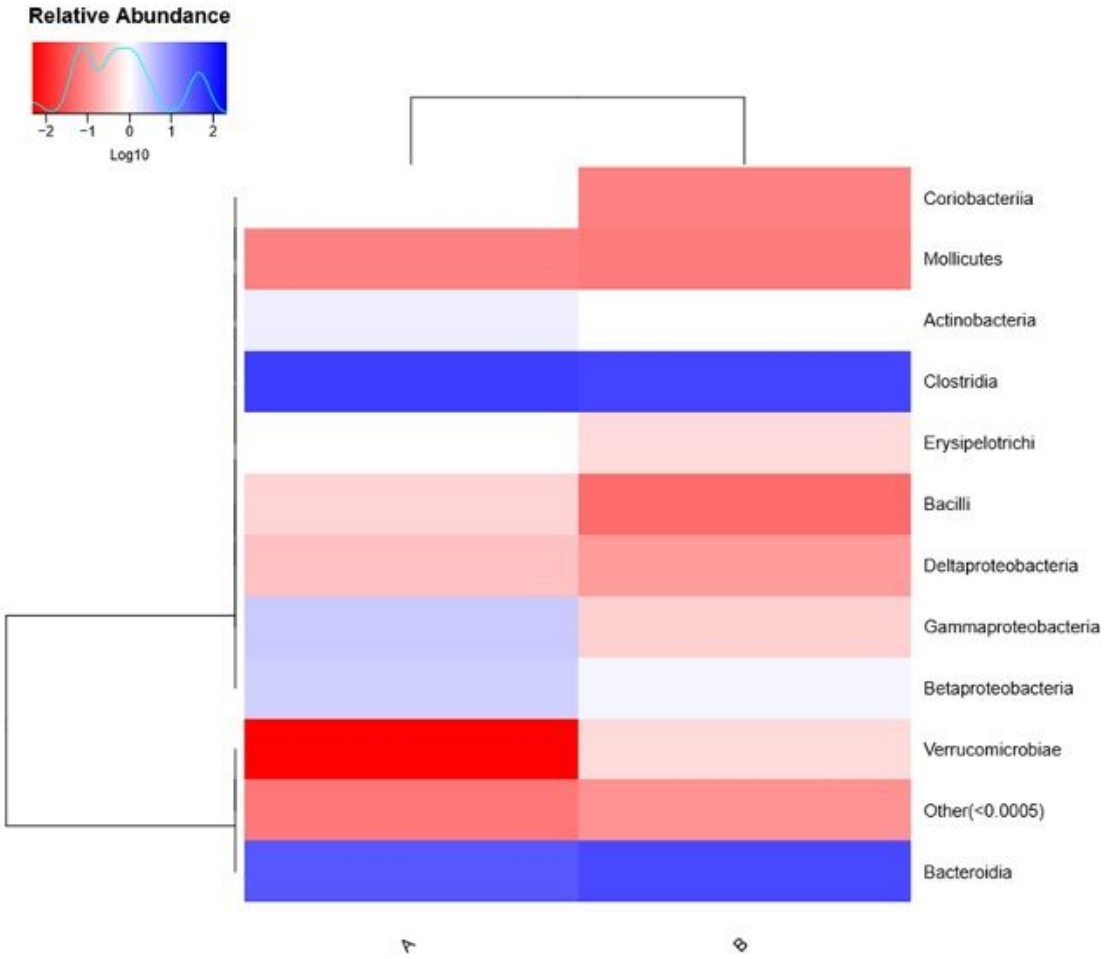

Figure 3

The Species abundance heat map about relative abundance of different flora. *Species abundance heat map is a graphical display method of clustering according to the similarity of species or sample abundance by the color gradient representing the relative abundance of species. (A: control group, internal medicine nurses; B: experimental group, pediatric nurses)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.docx