Characteristics of nasal and intestinal microbiota and effects of probiotics intervention in highly exposed people with PM2.5: a randomized, double-blind, placebo-controlled clinical study

Yongcan Wu
   Chengdu University of Traditional Chinese Medicine

Caixia Pei
   Chengdu University of Traditional Chinese Medicine

Xiaomin Wang
   Chengdu University of Traditional Chinese Medicine

Mingjie Wang
   Chengdu University of Traditional Chinese Medicine

Demei Huang
   Chengdu University of Traditional Chinese Medicine

Wei Xiao
   Chengdu University of Traditional Chinese Medicine

Fei Wang
   Chengdu University of Traditional Chinese Medicine

Zhenxing Wang (✉ wangzhenxing@vip.tom.com)
   Chengdu University of Traditional Chinese Medicine   https://orcid.org/0000-0001-6832-9652

Abstract

Background: Extended exposure to high concentrations of PM2.5 changes the human microbiota profile, which in turn may increase morbidity and mortality due to respiratory system damage. A balanced microecosystem is crucial to human health, and certain health-related problems may be addressed by effective microecosystem regulation. Recent studies have confirmed that probiotics may reduce the incidence of respiratory diseases. However, few studies have investigated probiotic treatment outcomes in subjects exposed to high concentrations of PM2.5.

Methods: This study is designed as a prospective, 2×2 factorial, randomized, participants- and assessor-blinded, placebo-controlled trial. One hundred and twenty eligible volunteers recruited from October 2019 to July 2020 in downtown Chengdu, China will be treated with either probiotics or placebo over 4 consecutive weeks. The primary outcome will be 16SrRNA sequencing assay data from nasal and intestinal secretions. Secondary outcomes will be pulmonary function, score on a gastrointestinal symptom rating scale, COOP/WONCA charts, and the Short-Form Health Survey 36 for quality of life. Results will be analysed to assess differences in clinical efficacy between groups. Six-month follow-up examinations will evaluate the long-term value of probiotics on cardiovascular and respiratory disease end-point events.

Discussion: We will explore the characteristics of nasal and intestinal microbiota in a population with high exposure to PM2.5. Probiotics and placebo interventions will be tested for efficacy in microbial balance regulation, effects on lung and physical functions, and quality of life improvement. This study is expected to provide reliable evidence to support the widespread promotion of probiotics in clinical practice for the protection of individuals with high exposure to PM2.5.

Background And Rationale

Due to the rapid development of industrialization and urbanization in China, air pollution has become one of the main risk factors for diseases among Chinese citizens [1]. In particular, the recent and frequently occurring hazy weather caused by PM2.5 has seriously affected the health and quality of life of the population [2, 3]. PM (fine particulate matter) is the general name for solid and liquid particles suspended in ambient air [2, 4]. Particle size is the most important factor in air pollution, and PM2.5 is a fine particle with a size of ≤ 2.5 μm. PM2.5 may adsorb a variety of organic compounds, heavy metals, pathogenic microorganisms, and acid oxides, and may enter the lower respiratory tract during respiratory movement, reaching the alveoli and possibly the blood circulation. These characteristics implicate PM2.5 as a potential cause of the recent increase in respiratory disease incidence [5].

Numerous epidemiological studies have revealed that the increase of PM2.5 concentration is closely correlated to the hospitalization rate and mortality associated with respiratory diseases [6–9]. High concentrations of environmental particles carry a large number of microorganisms, including many pathogens and opportunistic pathogens [10, 11]. Particulate matter is one of the key factors affecting the

airborne bacterial concentration and community structure [12, 13]. Several studies demonstrated that high-levels of PM2.5/PM10 were related to alterations in the human pharyngeal [14], nasal [15], and intestinal [16] microbiota composition. Long-term exposure to PM2.5 as the main component in air pollution disrupts the inherent balance of the human microbiota, and may induce and aggravate respiratory diseases.

The Food and Agriculture Organization of United Nations/World Health Organization (FAO/WHO) Expert Committee has defined probiotic strains as "live microorganisms which, when consumed in appropriate amounts in food, confer a health benefit on the host" [17]. The main probiotic component selected for this clinical trial is Lactobacillus rhamnose GG (LGG). Studies confirmed that LGG may regulate the human microbiota, positively affects immunity, has anti-inflammatory properties, and produces a biofilm that can mechanically protect the mucosa [18]. However, few studies have investigated these positive effects on individuals exposed to high concentrations of PM2.5. A randomized, double-blind controlled clinical trial will be conducted in this study to investigate the characteristics of nasal and intestinal microbiota, and the preventive and therapeutic effects of probiotics in healthy subjects exposed to an environment containing PM2.5. We hope to provide a clinical basis for the prevention and treatment of respiratory and circulatory diseases based on the study results.

Methods

Study design

This study incorporates a randomized, double-blind, placebo-controlled clinical trial and was developed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Statement (the SPIRIT checklist is shown in Additional file 1). The nasal and intestinal secretions of subjects exposed to PM2.5 will be collected and analysed to determine the characteristics of nasal cavity and intestinal flora under high PM2.5 exposure, and to investigate the clinical efficacy of probiotic intervention. A flowchart of this trial procedure is shown in Fig. 1. The Chinese Ethics Committee of Registering Clinical Trials (Ethical Review No.: ChiECRCT20190173), approved all the methods and ethics in the present study, and all procedures are in accordance with the Declaration of Helsinki v.08. Participants will sign a written informed consent form (see Additional file 2) with a clear understanding of the purpose, study procedure, and all potential risks related to the trial. All written informed consent forms will be collected and provided to members of the experimental group.

Participant recruitment

We will recruit 120 candidates. The recruitment methods used in this study include: (1) face-to-face communication, (2) bulletin boards, and (3) posters. Any eligible participant can contact our researchers using the provided telephone number and undergo screening to enter the group. Only those who fully meet the selection criteria, and sign the written informed consent form, will be registered and randomly assigned to placebo or probiotic treatment. All participants will be required to undergo a routine physical

examination and complete the relevant questionnaire. All subject personal information and data will be considered confidential. Only the members of the experimental group and the principal investigator will have access to the database. Participant enrolment will begin in October 2019, and end in July 2020.

Sample size

The recommended sample size was estimated based on the principal results of subject flora composition. To our knowledge, there have been no previous studies on the effects of probiotics on PM2.5-related components of flora. Therefore, the study design incorporating 120 participants resulted from sample size calculations for the primary outcomes using noninferiority tests utilizing PASS version 15.0 (NCSS, LLC, Kaysville, USA) software. We set the significance level to 0.05 with 80% power, and estimated the noninferior margins and standard deviations of primary outcomes on the basis of data from several similar interventional studies including patients with different diseases, e.g. diabetes [19, 20], and reflux esophagitis [21].

Selection criteria

Inclusion criteria for high-exposure population

We intend to include patients aged 22–65 with the following characteristics:

1. Male and female sanitation workers or traffic polices working in an environment with traffic pollution in Chengdu, for ≥3 years, and fixed working time ≥8 hours;
2. No history of gastroenteritis in the past month;
3. No hormones or antibiotics were used in the past month;
4. No nasal spray or other probiotics (such as lactic acid bacteria, Bifidobacterium, etc.), yoghurt or beverage used in the past 3 months;
5. No lymph node enlargement, inflammation, polyps, mass, runny nose, etc after clinical examination of lips, throat, ears, nostrils, and neck (especially anterior nostril);
6. No chronic constipation (defecation < 3 times a week), and haemorrhoids.

Inclusion criteria for low-exposure population

We intend to include patients aged 22–65 with the following characteristics:

1. Male and female staff of Chengdu Urban Environmental Hygiene Administration Bureau and Chengdu Traffic Administration Bureau, working indoors (with air conditioning), for ≥3 years, and working time ≥8 hours;
2. No history of gastroenteritis in the past month;

3. No hormones or antibiotics used in the past month;

4. No nasal spray or other probiotics (such as lactic acid bacteria, Bifidobacterium, etc.) yoghurt or beverage used in the past 3 months;

5. No lymph node enlargement, inflammation, polyps, mass, runny nose, etc after clinical examination of lips, throat, ears, nostrils, and neck (especially anterior nostril);

6. No chronic constipation (defecation < 3 times a week), and haemorrhoids.

Exclusion criteria

Criteria of excluded participants:

1. History of gastrointestinal surgery;

2. Long history of drug use;

3. History of smoking;

4. Pregnancy and lactation;

5. Could not understand and cooperate with the experimental process;

6. Participated in other clinical trials within the past month.

Randomization and allocation concealment

The random number will be generated by the BMI SPSS Statistics 24.0 software. Given the number of seeds, 60 subjects will be randomly assigned to receive treatment (therapeutic drugs and control drugs) in the high- and low-exposure groups, respectively. Professional statisticians will generate the random allocation table and grouping records. The random distribution table will be created in quadruplicate, one each by the principal investigator in charge of the project (WF), the project supervisor (WZX), the pharmacist (ZC), and the statistician (WZC).

Blinding

During the course of the experiment, neither the participants nor the researchers will have the grouping information. The participants will be divided into a treatment group, and a control group. According to the stratification of high- and low-exposures, the drug number, label, and packaging for each subject will be compiled from 001 to 060, respectively. The random numbers will be sealed in double opaque envelopes and managed by the researcher (LSQ) in charge of the blinding method. Each subject will be equipped with a corresponding emergency letter and the serial and drug number on the cover and stationery will be confirmed consistent with the label on the drug package. The test name, subject serial number, the group that corresponds with the test, and the specific drugs used will be indicated in the letter. In case serious

adverse events occur during the medical process, the envelope will be opened to break the blinding in an emergency, and should be kept in reserve by the researcher.

Trial procedure

Treatment providers

Four qualified clinical doctors of Traditional Chinese Medicine (TCM) with significant clinical experience (WYC, WMJ, WXM, PCX), will conduct a physical examination and pre-group assessment for the participants. All clinicians will complete a training session held by the principal investigator (WF) elaborating the procedure for recording the details of every treatment on individual Case Report Forms (CRFs), follow-up with participants, deal with adverse events, and obtain hands-on practical training.

Treatment regimen

Subjects who meet the inclusion criteria will be randomly assigned to the placebo and probiotic groups at 1:1.

1. Probiotics group: the same batch of Culturelle capsules provided by American i-Health (Cromwell, CT, USA) will be swallowed with warm water at < 36°C, one tablet at a time, once per day, over the test period of 4 weeks. The follow-up period will be 24 weeks. All drugs will be stored in a 4°C refrigerator.
2. Placebo group: the same batch of placebo made by placebo Experimental Center, School of Pharmacy, Chengdu University of TCM, and also will be swallowed with warm water at < 36°C, one tablet at a time, once per day, over the test period of 4 weeks. The follow-up period will be 24 weeks. All drugs will be stored in a 4°C refrigerator.
3. The probiotics group and placebo group capsules will be packaged with the same label, each box containing a 4week dose, a clearly visible label on each package stating "trial only", and other information including name, dose, administration schedule, storage condition indication, expiration date, and manufacturer name. The reception, handling, storage, and distribution of drugs will be the responsibility of Dr. Zhang Chen, a pharmacist.

Data and sample collection

The research data will be collected and managed using the Chinese Clinical Research Public Management platform (Res Man). Res Man is an electronic data collection and management system that records the management process for clinical trials, subject baseline data recorded during the trial, result data and other related data based on the Internet, and transmits all to the central database for preservation and management. The experimental data may only be accessed and operated by the

research team. The principal investigator will have access to real-time data but cannot make any changes to it.

All data collection staff will be trained on managing research questionnaires and evaluating body measurements, in accordance with the standard research programmes to ensure the high quality and consistency of the questionnaire. The quality of life evaluation scale Short-Form Health Survey 36(SF–36) [22], COOP/WONCA charts [23], and gastrointestinal symptom rating scale (GSRS), will be completed before entering the group, after the final treatment, 12 weeks after the final treatment and 24 weeks after the final treatment. The respiratory and circulatory disease follow-up records will be acquired 12 weeks after treatment, and 24 weeks after final treatment.

The pulmonary function data will be collected by respiratory specialists to ensure consistency and examinations will be performed before entering the group, after the final treatment, 12 weeks after the final treatment and 24 weeks after the final treatment.

The procedure for subject nasal specimen collection will include wiping the surface of nasal mucosa with sampling paper and immediately placing it in an aseptic cryopreservation tube. After the samples of the left and right nasal cavity are collected, they will be combined into one sample. After samples are collected, they will be sealed in a self-sealing bag, placed on dry ice, transported to the laboratory within 2 hours, and stored at −80°C.

The subject intestinal samples will be collected by using the faecal collection bowl (to avoid urine mixing). Subsequently, the samples will be dipped with sterile cotton swabs and transferred to an aseptic freezing tube, frozen in liquid nitrogen for more than 4 hours, and then transferred to −80°C for preservation.

All samples will be destructed after use.

Outcome measures

The assessment included flora analysis of nasal and intestinal secretions and questionnaires from researchers (all Chinese versions). Assessments will be started before allocation at screening (T0, baseline), 4 weeks after the final treatment (T1, primary endpoint), 12weeks after the final treatment (T2, secondary endpoint), and 24weeks after the final treatment (T3, final endpoint). The timeframe of data collection and assessments is shown in Table1 (the SPIRIT figure).

Primary outcome measure

The primary outcome measure will be the change in high-throughput sequencing 16SrRNA data from nasal and intestinal secretions. The samples will be collected before admission, and 4 weeks after drug intervention.

Secondary outcome measures

Secondary outcome measures include the following:

1. The subject health will be assessed with a concise SF−36 questionnaire and a COOP/WONCA Chart will be generated. The subject quality of life will be summarized on eight aspects: physical function, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health.
2. The gastrointestinal subject function will be evaluated with the score from the GSRS.
3. A pulmonary function test will be performed to evaluate the influence of PM2.5 on subject pulmonary function.
4. The long-term efficacy of probiotics will be evaluated by recording a follow-up table of cardiovascular and respiratory diseases end-point events.

Adverse events reporting and safety monitoring

The Culturelle capsule used in this trial is a commercially available dietary supplement, therefore the safety risk of the participants may be controlled, and the Chinese Ethics Committee of Registering Clinical Trials (Ethical Review No.: ChiECRCT20190173) has approved the study. We will ensure that every participant is aware of common adverse events related to the treatment in this study (such as indigestion or diarrhoea), as well as serious adverse events (classified as life-threatening, permanently incapacitating, or requiring hospitalization) through the written consent form and detailed face-to-face consultations. During the treatment, participants must report any adverse events they may experience to the project team members, who must also record any adverse events they may have observed. All adverse events will be collected and presented promptly in detail to the project supervisor, who will determine the subsequent handling (including intimate observation, additional medical management, or early termination of participation), and record both the adverse events and the final outcomes in the CRF.

Data management and monitoring

Data collection and monitoring will be managed by a specialized data and safety monitoring board (DSMB). The DSMB is composed of a statistician, a deputy chief physician in the respiratory department, and a junior Chinese medicine practitioner, and will be established before the first enrolment of participants. The DSMB will be free to investigate all participant information, and has no prior competitive interest with other members of the experimental group. The project team members will collect and record the original subject data with the CRFs, including a brief medical record, basic information, treatment records, pre- and post-treatment evaluation data, follow-up data, adverse event records, etc. Any changes to these paper-based data forms will not be allowed without the investigation and authorization of the DSMB. Two team members (PCX, WMJ) blinded to the allocation will responsible for copying

information into a custom-designed and password-protected database on the Res Man Research Manager of the Clinical Trial Management Public Platform.

Adherence to study interventions

We will use several strategies to encourage and monitor compliance with research interventions. Clear oral and written instructions will be provided during the 7-month period from the beginning of the screening, to the end of the follow-up. The content will include guidance on lifestyle assessment, and adherence to daily research capsule intake. Two weeks after the beginning of the study, all participants will be contacted to assess how they manage interventions and, if necessary, further personalized guidance will be provided. All unused medicines will be returned and documented.

Statistical methods

The study will adopt different statistical methods according to the various types of detection data.

1. Community composition analysis: R language software (R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org/) will be utilized for a variety of data conversions. We will use the ggplot2 package (https://ggplot2.tidyverse.org/) to draw a diagram.

2. Alpha diversity analysis: will be performed with R language software. The PD index will be calculated using the picante package (https://CRAN.R-project.org/package = picante), other indices will use the vegan package (https://CRAN.R-project.org/package = vegan). The Wilcoxon rank sum test will use the wilcox.test function of the stats package (https://www.rdocumentation.org/packages/stats/versions/3.6.1/topics/wilcox.test), and the Kruskal-Wallis rank sum test (https://stat.ethz.ch/R-manual/R-devel/library/stats/html/kruskal.test.html) of more than two groups will use the kruskal.test function. Multiple comparisons will use the agricolae package (https://CRAN.R-project.org/package = agricolae).

3. Beta diversity analysis: will be performed with R language software. The Unifrac distance will be calculated using the GUniFrac package (https://CRAN.R-project.org/package = GUniFrac), and the Bray-Curtis and Jaccard distances will be calculated using the vegdist function of the vegan package. PCoA analysis will use the ape package (https://CRAN.R-project.org/package = ape). PCA and NMDS analysis will use the vegan package. Cluster analysis will use the hclust function of the stats package. Anosim, MRPP, and PerMANOVA will be calculated using the anosim function, mrpp function, and adonis functions of the vegan package, respectively.

4. Differential species analysis: LEfSe analysis will be performed with Python's LEfSe package (https://www.python.org/). Random forest analysis will use the R language random forest package, and metastats analysis will be performed with R language software.

5. Correlation analysis will use the cor.test function of the R language stats package.

6. The significance level of all difference tests will be set to 0.05.

7. Baseline, score table and lung function statistical methods: will be performed with the SPSS24.0 statistical software for statistical analysis and processing, the significance level will be set to 0.05. The measurement data will be expressed as "x ±s", the Student's group t test will be used to compare groups satisfying the normality and homogeneity of variance, and the Mann-Whitney U test will be used for those that did not conform to the normal distribution and the homogeneity of variance.

Discussion

Exposure to PM2.5 can significantly increase the risk of respiratory and circulatory diseases. Clinical epidemiological evidence has demonstrated that symptoms of chronic bronchitis and abnormal pulmonary function are closely related to exposure to PM2.5 [24, 25]. Both short-term and long-term exposure to PM2.5 can directly lead to an increase in the incidence of respiratory diseases, clinical consultation rates, and hospitalization rates [26–28]. The microorganisms carried by PM2.5 induce the pro-inflammatory response of resident immune cells, increase intestinal permeability, and change the lumen environment of the intestine, leading to growth of specific microbial strains better suited for survival in an inflammatory environment. These changes in the microenvironment will alter the intestinal microbiota of the host [29–31].

Since few studies currently exist for reducing the toxicological effects of PM2.5, any additional effective interventions would be very valuable. It is accepted that the microecosystem plays a significant role in health, and interventions to modify the microbiota are demonstrating value for treatment of several conditions related to individual health. Probiotics form the basis of the interventions in this study, and have the potential to address specific health concerns. The purpose of this study is to evaluate the protective effect of probiotics in subjects with high exposure to PM2.5, and to investigate possible microbial molecular mechanisms. The study results should provide valuable information regarding health maintenance and pharmaceutical intervention of probiotics in individuals with high exposure to PM2.5.

Trial Registration

The trial was pre-registered at the China Clinical Trials Registry on August 27, 2019 under the registration number ChiCTR1900025469. See http://www.chictr.org.cn/index.aspx

Trial Status

This paper is based on protocol version 2.0 dated 25 September 2019. Recruitment began in October 2019, and approximate date of completion is July 2020. Any major protocol changes will be notified to the ethics committee and updated on the Chine

Abbreviations

CRF: Case Report Forms; DSMB: Data and safety monitoring board; FAO: Food and Agriculture Organization; GSRS: Gastrointestinal symptom rating scale; LGG: Lactobacillus rhamnose GG; PM: Fine particulate matter; Res Man: Research Public Management platform; SF−36: Short-Form Health Survey 36; SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials; TCM: Traditional Chinese Medicine; WHO: World Health Organization.

Declarations

Acknowledgements

Authors' contributions

The trial was designed by WZX and WYC, and improved by WF and XW. WYC drafted the manuscript, which was carefully revised, edited, and discussed by WZX, PCX, WMJ, and WXM. All authors read and approved the final manuscript.

Funding

Availability of data and material

Data sharing is not applicable to this article as no datasets are reported. Availability of datasets generated in the study will be included in papers reporting study outcomes. Access to the full protocol and model consent forms may be available from the author upon reasonable request.

Ethics approval and consent to participate

The Chinese Ethics Committee of Registering Clinical Trials reviewed this study protocol and gave its approval and consent on 16 September 2019 (Ethical Review Number: ChiECRCT20190173). We ensure that written informed consent will be obtained from all study participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

All authors：Hospital of Chengdu University of Traditional Chinese Medicine：No.39 Shi-er-qiao Road, Chengdu, 610072, Sichuan Province, P. R. China.

References

1.Hu LW, Lawrence WR, Liu Y, Yang BY, Zeng XW, Chen W, Dong GH: Ambient Air Pollution and Morbidity in Chinese. Adv Exp Med Biol2017, 1017:123–51.

2.Liu C, Chen R, Sera F, Vicedo-Cabrera AM, Guo Y, Tong S *et al:* Ambient Particulate Air Pollution and Daily Mortality in 652 Cities. N Engl J Med2019, 381(8):705–15.

3.Stanaway JD, Afshin A, Gakidou E, Lim SS, Abate D, Abate KH *et al:* Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet2018, 392(10159):1923–94.

4.Morakinyo OM, Mokgobu MI, Mukhola MS, Hunter RP: Health Outcomes of Exposure to Biological and Chemical Components of Inhalable and Respirable Particulate Matter. Int J Environ Res Public Health2016, 13(6).

5.WHO's global air-quality guidelines. Lancet2006, 368(9544):1302.

6.Wang L, Zhong B, Vardoulakis S, Zhang F, Pilot E, Li Y, Yang L, Wang W, Krafft T: Air Quality Strategies on Public Health and Health Equity in Europe-A Systematic Review. Int J Environ Res Public Health2016, 13(12).

7.Lu F, Xu D, Cheng Y, Dong S, Guo C, Jiang X, Zheng X: Systematic review and meta-analysis of the adverse health effects of ambient PM2.5 and PM10 pollution in the Chinese population. Environ Res2015, 136:196–204.

8.Ren M, Fang X, Li M, Sun S, Pei L, Xu Q, Ye X, Cao Y: Concentration-Response Relationship between PM2.5 and Daily Respiratory Deaths in China: A Systematic Review and Metaregression Analysis of

Time-Series Studies. Biomed Res Int2017, 2017:5806185.

9.Cai J, Peng C, Yu S, Pei Y, Liu N, Wu Y, Fu Y, Cheng J: Association between PM2.5 Exposure and All-Cause, Non-Accidental, Accidental, Different Respiratory Diseases, Sex and Age Mortality in Shenzhen, China. Int J Environ Res Public Health2019, 16(3).

10.Cao C, Jiang W, Wang B, Fang J, Lang J, Tian G, Jiang J, Zhu TF: Inhalable microorganisms in Beijing's PM2.5 and PM10 pollutants during a severe smog event. Environ Sci Technol2014, 48(3):1499–507.

11.Ji L, Zhang Q, Fu X, Zheng L, Dong J, Wang J, Guo S: Feedback of airborne bacterial consortia to haze pollution with different PM2.5 levels in typical mountainous terrain of Jinan, China. Sci Total Environ2019, 695:133912.

12.Li W, Yang J, Zhang D, Li B, Wang E, Yuan H: Concentration and Community of Airborne Bacteria in Response to Cyclical Haze Events During the Fall and Midwinter in Beijing, China. Front Microbiol2018, 9:1741.

13.Liu H, Zhang X, Zhang H, Yao X, Zhou M, Wang J *et al:* Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter. Environ Pollut2018, 233:483–93.

14.Qin T, Zhang F, Zhou H, Ren H, Du Y, Liang S *et al:* High-Level PM2.5/PM10 Exposure Is Associated With Alterations in the Human Pharyngeal Microbiota Composition. Front Microbiol2019, 10:54.

15.Gao X, Xu Y, Cai Y, Shi J, Chen F, Lin Z *et al:* Effects of filtered fresh air ventilation on classroom indoor air and biomarkers in saliva and nasal samples: A randomized crossover intervention study in preschool children. Environ Res2019, 179(Pt A):108749.

16.Alderete TL, Jones RB, Chen Z, Kim JS, Habre R, Lurmann F, Gilliland FD, Goran MI: Exposure to traffic-related air pollution and the composition of the gut microbiota in overweight and obese adolescents. Environ Res2018, 161:472–8.

17.Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. In. Edited by Organization FaAOaWH. http://www.fao.org/tempref/docrep/fao/meeting/009/y6398e.pdf.: Accessed 16 July 2019.

18.Capurso L: Thirty Years of Lactobacillus rhamnosus GG: A Review. J Clin Gastroenterol2019, 53 Suppl 1:S1-S41.

19.Alokail MS, Sabico S, Al-Saleh Y, Al-Daghri NM, Alkharfy KM, Vanhoutte PM, McTernan PG: Effects of probiotics in patients with diabetes mellitus type 2: study protocol for a randomized, double-blind, placebo-controlled trial. Trials2013, 14.

20.Stefanaki C, Michos A, Mastorakos G, Mantzou A, Landis G, Zosi P, Bacopoulou F: Probiotics in Adolescent Prediabetes: A Pilot RCT on Glycemic Control and Intestinal Bacteriome. J Clin Med2019, 8(10).

21.Sun QH, Wang HY, Sun SD, Zhang X, Zhang H: Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial. World J Gastroenterol2019, 25(17):2110–21.

22.Maruish ME E: User's manual for the SF−36v2 health survey. 3rd ed. QualityMetric Incorporated; 2011.

23.van Weel C K-ZC, Touw-Otten FWMM, van Duijn NP: Meyboom-de Jong B. Measuring Functional Health Status With the COOP/WONCA Charts. A Manual. Groningen, The Netherlands: WONCA, ERGHO, and NCH-University of Groningen1995.

24.Nightingale R, Lesosky M, Flitz G, Rylance SJ, Meghji J, Burney P, Balmes J, Mortimer K: Noncommunicable Respiratory Disease and Air Pollution Exposure in Malawi (CAPS). A Cross-Sectional Study. Am J Respir Crit Care Med2019, 199(5):613–21.

25.Hao Y, Balluz L, Strosnider H, Wen XJ, Li C, Qualters JR: Ozone, Fine Particulate Matter, and Chronic Lower Respiratory Disease Mortality in the United States. Am J Respir Crit Care Med2015, 192(3):337–41.

26.Xie J, Teng J, Fan Y, Xie R, Shen A: The short-term effects of air pollutants on hospitalizations for respiratory disease in Hefei, China. Int J Biometeorol2019, 63(3):315–26.

27.Nhung NTT, Amini H, Schindler C, Kutlar Joss M, Dien TM, Probst-Hensch N, Perez L, Künzli N: Short-term association between ambient air pollution and pneumonia in children: A systematic review and meta-analysis of time-series and case-crossover studies. Environmental Pollution2017, 230:1000–8.

28.Luo L, Zhang Y, Jiang J, Luan H, Yu C, Nan P, Luo B, You M: Short-Term Effects of Ambient Air Pollution on Hospitalization for Respiratory Disease in Taiyuan, China: A Time-Series Analysis. Int J Environ Res Public Health2018, 15(10).

29.Salim SY, Kaplan GG, Madsen KL: Air pollution effects on the gut microbiota: a link between exposure and inflammatory disease. Gut Microbes2014, 5(2):215–9.

30.Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR, Vavricka SR, Fiocchi C: Environmental triggers in IBD: a review of progress and evidence. Nat Rev Gastroenterol Hepatol2018, 15(1):39–49.

31.Salim SY, Jovel J, Wine E, Kaplan GG, Vincent R, Thiesen A, Barkema HW, Madsen KL: Exposure to ingested airborne pollutant particulate matter increases mucosal exposure to bacteria and induces early onset of inflammation in neonatal IL−10-deficient mice. Inflamm Bowel Dis2014, 20(7):1129–38.

Additional Files

Additional file 1: The SPIRIT Checklist (DOC 125kb).

Additional file 2: Informed consent materials⬜DOC 17kb⬜

Table

Due to technical limitations, Table 1 is available in the Supplementary Files.

Figures

Figure 1

Study process: flowchart of study procedure

Supplementary Files

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

- InformedConsentForm.docx
- Table1.docx
- SPIRITchecklist.doc