

A Mendelian Randomization Study:
Roles of Gut Microbiota in Sepsis – Who is the Angle?

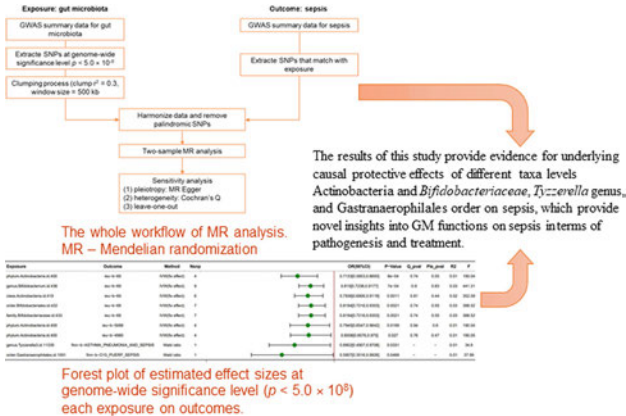
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Abstract

Gut microbiota (GM) is a crucial underlying player during sepsis pathogenesis. However, the causal relationship is unclear and remains to be determined. A two-sample Mendelian randomization study was implemented. The statistical data about sepsis together with GM summarized from genome-wide association studies were evaluated. Instrumental variables were defined as single-nucleotide polymorphisms with prominent correlations with exposure. The inverse-variance-weighted test was employed as a major approach of Mendelian randomization analysis to estimate of causal relationships. The inverse-variance-weighted analysis results demonstrated that at different taxa levels, Actinobacteria and *Bifidobacteriaceae* influence sepsis. Actinobacteria had negative relationships to sepsis risk at the phylum ($\beta = -0.34$, $SE = 0.10$, $p = 0.0008$) and class ($\beta = -0.23$, $SE = 0.07$, $p = 0.0011$) levels in outcome coded ieu-b-69. Actinobacteria at the phylum level ($\beta = -0.22$, $SE = 0.10$, $p = 0.027$) was also negatively associated with sepsis in outcome coded ieu-b-4980. *Bifidobacteriaceae* at the order ($\beta = -0.20$, $SE = 0.06$, $p = 0.0021$), family ($\beta = -0.20$, $SE = 0.06$, $p = 0.0021$), and genus ($\beta = -0.20$, $SE = 0.06$, $p = 0.0007$) levels were all negatively correlated with the risk of sepsis in outcome coded ieu-b-69. The results of the Wald ratio model showed that *Tyzzereella* genus (OR (95%CI)



= 0.6902[0.4907,0.9708], $p = 0.0331$) and Gastraerophilales order (OR (95%CI) = 0.5907[0.3516,0.9926], $p = 0.0468$) were negatively connected with sepsis. This study implied at different taxa levels Actinobacteria and *Bifidobacteriaceae*, *Tyzzereella* genus, and Gastraerophilales order have a causal relationship with sepsis, indicating that they are protective factors for the incidence of sepsis.

Key words: causal relationship, gut microbiota, Mendelian randomization, sepsis

Introduction

Sepsis refers to a lethal organ dysfunction that is attributed to the dysregulated responses of the host to infections, serving as a leading reason for morbidity and mortality worldwide, so the World Health Organization has recognized it as a global health emergency. The pathogenesis of sepsis remains unclear to date (Anggraini et al. 2022), including imbalance of inflam-

matory response, immune dysfunction, coagulation dysfunction, and other aspects. The process of its onset is a gradual sequential reaction, and further development may lead to the development of multiple organ dysfunction syndrome. The gastrointestinal tract is the most unique among them. On the one hand, the gastrointestinal tract is the organ first affected by sepsis, and it is affected by both endogenous and exogenous factors, leading to functional disorders. On the other hand, the

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gastrointestinal tract can act as an intermediate hub, causing damage to other distant organs and accelerating the progression of sepsis (Longhitano et al. 2020).

The microbiota distributed in the gastrointestinal tract is commonly referred to as the gut microbiota (GM), which exerts an essential effect on the maintenance of physiological functions such as metabolism, immunity, and endocrine in the human body (Klingensmith and Coopersmith 2023). There is increasing evidence that the imbalance of GM leads to a higher risk of sepsis and worse outcomes (Adelman et al. 2020; Kang and Thomas 2021; Kullberg et al. 2021; Miller et al. 2021; Niu and Chen 2021). Recent evidence has demonstrated that gut microbiota regulates host physiological homeostasis mediators. Therefore, maintaining or restoring microbiota and metabolite composition might be a therapeutic or prophylactic target against critical illness (Niu and Chen 2021). Fecal microbiota transplantation and supplementation of probiotics are microbiota-based treatment methods that are limited in evidence-based efficacy. The detailed roles of these microorganisms in the sepsis pathophysiology need further investigation.

The causality involving outcomes together with exposure was probed into through a Mendelian randomization (MR) study with instrumental variables (IVs) selected from single-nucleotide polymorphisms (SNPs). Compared to randomized controlled trials, MR analysis can more quickly and more economically determine the cause and effect involving exposure factors plus outcomes (Skrivankova et al. 2021). The correlation of GM with sepsis has not been investigated using MR analysis

so far. Therefore, the causal relationship between GM and sepsis, as well as the concrete functions of diverse GM taxa in sepsis, deserves in-depth investigation. Therefore, this MR study was performed by virtue of massive summary statistics from genome-wide association studies (GWASs) concerning GMs and sepsis to distinguish GM taxa with possible influences on sepsis to give support to current evidence while providing novel opinions for the treatment plus prevention of sepsis.

Experimental

Materials and Methods

Study design. A two-sample MR study was carried out to exploit the association of GM with sepsis. Fig. 1 illustrates the whole design of our research.

Data sources. The MiBioGen study provided GM-related GWAS data (Kurilshikov et al. 2021), a multi-ethnic genome-wide meta-analysis on GM with most enormous scale. In this study, the sequencing profiles for 16S rRNA gene targeting the variable regions such as V4, V3–V4, and V1–V2, together with the data of whole-genome genotyping collected from 18,473 individuals (with a predominant proportion of European ancestry) in 25 cohorts, were utilized. Finally, 122,110 host genetic variants were obtained from GWASs, which were mapped to the abundance-associated genetic loci in 211 taxa (16 classes, 131 genera, 20 orders, 9 phyla, and 35 families) through the analysis

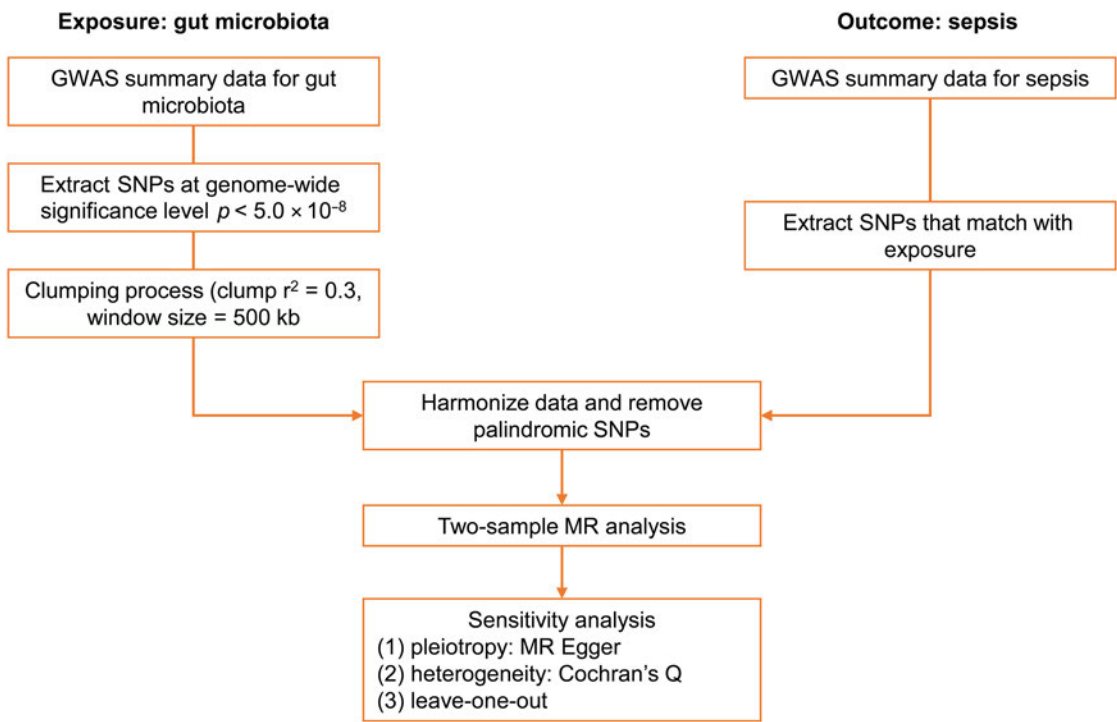


Fig. 1. The whole workflow of Mendelian randomization (MR) analysis.

Table I
Details of data sources.

	Trait	Population	Sample_ size	Case	Control
ieu-b-5088	Sepsis (under 75)	European	462,869	11,568	451,301
ieu-b-4980	Sepsis	European	486,484	11,643	474,841
ieu-b-69	Sepsis	European	462,918	10,154	454,764
finn-b-O15_PUERP_SEPSIS	Puerperal sepsis	European	121,441	2,286	119,155
finn-b-ASTHMA_PNEUMONIA_AND_SEPSIS	Asthma-related pneumonia or sepsis	European	140,994	5,545	135,449

of variations in GM taxa among diverse populations and GWAS analysis by taxa step by step.

MR-Base database (<https://www.mrbase.org>) (Hemani et al. 2018), which has incorporated the results of GWASs involving 605 traits in the UK Biobank so far, was utilized to extract the GWAS summary statistics for sepsis. It comprises 11 billion pieces of SNP-trait associations uncovered by 1673 GWASs, with regular updates. The cohorts in UK Biobank (<https://www.ukbiobank.ac.uk>) enrolled nearly 500,000 adults from the UK in 2006–2010. The participants were permitted to obtain extensive data on phenotypes and genotypes. According to different quality control standards, a total of three GWAS summary statistics of sepsis from MR-Base were obtained, including 2 GWAS that Hospital Episode Statistic coded only, performed using regenie v2.2.4 (Mbatchou et al. 2021) and 1 GWAS performed using MRC IEU UK Biobank pipeline. The ids are ieu-b-4980 with 11643 cases and 474841controls, ieu-b-5088 with 1896 cases and 484588 controls, ieu-b-69 with 10154 cases and 454764 controls, respectively. The hospital admission data were coded by ICD and linked to the UK Biobank to identify the sepsis cases, including ICD-10 codes A02, A40, A39, and A41. The whole participants are of European descent.

The other two from the FinnGen database (<https://finngen.gitbook.io/documentation>) (Kurki et al. 2023) were employed to offer the remaining summary statistics of GWAS. FinnGen is a large-scale genomic study in Finland aiming to create a comprehensive genetic and health data database from up to 500,000 participants. The project aims to identify new genetic targets for preventing and treating common diseases involving cancer, diabetes, and cardiovascular disease. This database is open to researchers worldwide and has already yielded important discoveries. The identifiers for their entries in MR-Base are finn-b-O15_PUERP_SEPSIS and finn-b-ASTHMA_PNEUMONIA_AND_SEPSIS, respectively (Table I).

Data processing. A procedure integrating distance <500 kb, European ancestry, and LD $r^2 < 0.3$ was applied to determine the GM-associated lead SNPs at the genome-wide significance level ($p < 5.0 \times 10^{-8}$). Then,

the same SNPs were picked up from GWAS summary statistics of sepsis to conduct subsequent MR analysis.

Two-sample MR. R-based “TwoSampleMR” package (version v0.5.6) (Hemani et al. 2018) was employed to accomplish the two-sample MR analysis, with five models in total, namely, MR-Egger, Wald ratio, weighted median, inverse-variance-weighted (IVW), and weighted mode established. The lead SNPs, after clumping, were employed as IVs. For the purpose of guaranteeing the robustness of data and the precision of results, the IVs for the models in this study were selected based on three major principles: 1) IVs had relationships to GM taxa ($p < 5.0 \times 10^{-8}$), 2) IVs were only associated with sepsis through their relation to risk factors, 3) there was no correlation between IVs and confounders. At last, SNPs were excluded in the case of linkage disequilibrium (clump = 500 kb, $r^2 = 0.3$).

Our study used the IVW approach as a primary analytical method when there were two or more instrumental variables, and the Wald ratio model was utilized for data analysis with merely one IV existing. If the IVW model produced a significant result, the GM taxa would be assumed to have a causal link with sepsis. The other three models were also utilized as references, and if the favorable outcomes were reproduced, we considered the results more reliable. Cochran Q test plus MR-Egger regression were employed to evaluate the heterogeneity and pleiotropic effect, respectively, to avoid violating MR assumptions. Causal effects were considered credible in the case of $p > 0.05$ for MR-Egger intercept, together with $p > 0.05$ for Cochran’s Q test. A random-effect IVW model was employed when significant heterogeneity ($p < 0.05$) emerged. Leave-one-out analysis was utilized to amine the promotion of individual SNP to the causal estimate, which was repeated by sequentially discarding the SNPs related to each exposure. In our study, the causality involving GM and sepsis was appraised by the combination of standard errors (SE), odds ratio (OR), β , and confidence interval (CI).

Statistical analysis. R 4.0.2 software (R Core Team 2020) was used for statistical analysis. *F*-statistic was evaluated to determine the presence or absence of weak IV bias, where the IVs based on genetic variations

Table II
Mendelian randomization (MR) analysis of gut microbiota on sepsis at genome-wide significance level ($p < 5.0 \times 10^{-8}$).

Classification	Bacterial taxa	Outcome	No. SNP	MR method	Beta	SE	OR	95% CI	p	F	Horizontal pleiotropy	Heterogeneity
Actinobacteria	phylum	ieu-b-4980	4	IVW	-0.222	0.1	0.801	0.658-0.975	0.027	190.05	0.47	0.76
			4	Weighted median	-0.245	0.116	0.783	0.623-0.983	0.035			
		ieu-b-5088	4	IVW	-0.23	0.099	0.795	0.655-0.964	0.02	190.04	0.6	0.94
			4	Weighted median	-0.25	0.109	0.779	0.629-0.965	0.022			
		ieu-b-69	4	IVW	-0.338	0.101	0.713	0.585-0.869	0.001	190.04	0.55	0.74
			4	Weighted median	-0.367	0.118	0.693	0.550-0.873	0.002			
<i>Bifidobacteriaceae</i>	order	ieu-b-69	6	IVW	-0.231	0.071	0.794	0.691-0.912	0.001	352.59	0.44	0.02
			6	Weighted median	-0.241	0.089	0.786	0.660-0.936	0.007			
		ieu-b-4981	7	IVW	1.051	0.368	2.861	1.391-5.885	0.004	390.71	0.18	0.3
			7	Weighted median	1.024	0.49	2.783	1.065-7.276	0.037			
		ieu-b-69	7	IVW	-0.199	0.065	0.819	0.722-0.930	0.002	388.52	0.55	0.74
			7	Weighted median	-0.208	0.081	0.812	0.692-0.952	0.01			
<i>Tyzzerella</i> Gastranaerophilales	genus order	ieu-b-69	7	IVW	-0.231	0.071	0.819	0.722-0.930	0.001	388.52	0.55	0.74
			7	Weighted median	-0.208	0.082	0.812	0.691-0.954	0.012			
		ieu-b-69	9	IVW	-0.204	0.065	0.815	0.724-0.912	0.002	441.31	0.83	0.9
			9	Weighted median	-0.226	0.079	0.798	0.683-0.931	0.004			
		finn-b-ASTHMA_PNEUMONIA_AND_SEPSIS	1	Wald ratio	-0.371	0.174	0.69	0.490-0.970	0.033	-	-	-
		finn-b-O15_PUERP_SEPSIS	1	Wald ratio	-0.526	0.265	0.59	0.352-0.992	0.047	-	-	-

Beta – estimated causal effect coefficient in different MR methods, CI – confidence interval, F – F-statistics, heterogeneity – p-value of heterogeneity analysis, horizontal pleiotropy – p-value of horizontal pleiotropy analysis, MR method – the type of model applied in MR analysis, No. SNP – number of SNPs, OR – odds ratio, p – p-value of causal estimation in different MR methods, SE – standard error of coefficient estimate

each exposure's predicted degree of impact on outcomes. There was no horizontal pleiotropy heterogeneity in addition to horizontal pleiotropy observed from IVs and outcomes. Furthermore, all *F*-statistics above 10 verified a low probability of a weak IV bias. The detailed results are presented in Table SII.

Discussions

The causal correlation of sepsis with GM was evaluated through two-sample MR analyses in this study, the first research using MR analyses to assess this relationship. We found that at different taxa levels, Actinobacteria and *Bifidobacteriaceae* are causally associated with low risk of sepsis, indicating their protective effect on sepsis. Other species of GM indicated potential protective effects against sepsis, including *Tyzzzeria* genus and Gastranaerophilales order.

Sepsis substantially threatens the public health due to its high morbidity and mortality. Unfortunately, over the past decades, antibiotics and supportive care represent the only established targeted therapy of sepsis (Evans et al. 2021). As shotgun metagenomic sequencing, 16S rRNA, and other culture-independent methods are emerging and developing (Durazzi et al. 2021), increasing evidence now suggests that the GM exerts crucial effects on sepsis from the aspect of the pathophysiological process (Chancharoenthana et al. 2023). Sepsis can significantly influence the composition, diversity, and balance of normal GM due to the pathophysiological changes such as gut hypoperfusion and impaired mucosal integrity and the clinical interventions, including antibiotics for treating sepsis. At the same time, its development may be facilitated by these factors in turn, forming a vicious cycle (Miller et al. 2021). GM alteration increases sepsis susceptibility before sepsis onset via multiple mechanisms, such as stimulating the pathogenic intestinal bacteria to expand, initiating strong proinflammatory responses of the immune system, and inhibiting beneficial microbial products (short-chain fatty acids and so on) from generation. Once sepsis is on set, GM disruption is exacerbated while the susceptibility to end-organ dysfunction is enhanced. According to finite evidence, the reduction of sepsis risk plus the improvement of sepsis outcomes may be attributed to selective decontamination of the digestive tract, prebiotics, fecal microbial transplantation, probiotics, and other microbiome-based therapies, while the safety of such therapies has been rarely considered (Adelman et al. 2020). Research on specific microbiome changes during sepsis and the mechanism of action on sepsis have therefore been identified as a critical problem to explore.

Belonging to the Actinobacteria phylum, Bifidobacteriales order, and *Bifidobacteriaceae* family

(Mancino et al. 2019), the *Bifidobacterium* genus was first discovered by Tissier in 1899 and then separated from feces of a breastfeeding infant (Ventura et al. 2014). As an essential bacterial genus composing the intestinal flora in human beings, *Bifidobacterium* has such physiological functions as anti-tumor activity, promotion of nutrient absorption, immune regulation, and maintenance of intestinal micro-ecological balance. *Bifidobacterium* is generally reduced in critical ill patients and related to prognosis. Wei et al. (2021) found that the reduced abundance of *Bifidobacterium* had preferable effects on predicting in-hospital mortality of critically ill patients. Consistent with previous findings, recently a study to assess the association between GM composition and Day-28 mortality in critically ill patients indicated that survivors had a higher abundance of *Bifidobacterium* (Prevel et al. 2022). There are similar changes in sepsis. Peng et al. (2023) found *Bifidobacterium* reduced in different intestinal segments of mice with sepsis by cecum ligation and puncture modeling. Sun et al. (2023) found that patients with sepsis showed a decreased abundance of *Bifidobacterium*, too. Previous randomized controlled trials and meta-analyses examined the impact of probiotics on sepsis, most containing *Lactobacillus* and *Bifidobacterium* species, which yielded complex results. As corroborated by a meta-analysis on critically ill patients, probiotics were associated with alleviated ventilator-associated pneumonia plus infections. There was heterogeneity of inclusion criteria and probiotic species selected for the included trials, in which merely one subset was established explicitly for sepsis patients (Manzanares et al. 2016). The clinical conditions of preterm neonates with low birth weight and the roles of oral supplementation of probiotics in influencing *Lactobacillus* and *Bifidobacterium* populations in the gut were examined through a randomized, multicenter, double-blind, placebo-controlled trial. They found that the probiotic bacteria selected and appropriately characterized are safe for preterm neonates to normalize the disturbed GM and conducive to lowering the incidence rate of staphylococcal sepsis (Robertson et al. 2020). Whether GM disruption before the onset of sepsis aggravates the prognosis of humans with sepsis has not been clarified. Our study suggests the protective effect of *Bifidobacterium* on sepsis through MR analysis, indirectly confirming the results of other studies and providing reliable evidence for future clinical randomized controlled studies.

We found that the Gastranaerophilales order was negatively connected with sepsis, but the mechanism is unclear. The Gastranaerophilales order lacks the genes essential for aerobic respiration so that it can grow in human and animal guts with low or nearly no oxygen. It can be classified as non-photosynthetic Cyano-

bacteria (Melainabacteria class) (Hu and Rzymiski 2022). The proportion of Cyanobacteria phylum in the GM is less than 1%. Di Rienzi et al. (2013) utilized the human gut and groundwater to assemble the whole genomes of non-photosynthetic cyanobacterium-like organisms for the first time in 2013. Even though the correlations of diverse diseases with the composition of human GM have been explored in numerous studies, the association between health and intestinal Cyanobacteria and Gastranaerophilales in humans should be researched in detail. In the past years, a couple of researches indicated that gut Cyanobacterial abundance increased in many diseases, such as amyotrophic lateral sclerosis (Di Gioia et al. 2020), neurodevelopment disorder (Zhang et al. 2021), acute gastroenteritis (Xiong et al. 2021), Wilson's Disease (Cai et al. 2020) and allergy rhinitis (Zhu et al. 2020). However, there is no observational study on the correlation between sepsis and Gastranaerophilales. We infer that the mechanism by which Gastranaerophilales participates in sepsis may be related to the metabolism of tryptophan, which belongs to the crucial amino acids supplied to human bodies by diets, containing the structure of an indole and metabolized by GM to produce indole and indole derivatives. Rosario et al. (2021) found Gastranaerophilales can contribute to increased concentrations of indole, which could be converted into indole-3-propionic acid (IPA). It is argued that IPA conjugates with the aromatic hydrocarbon receptor of intestinal epithelium cells or pregnane X receptor of lymphocytes to activate the immune system, thereby stimulating the intestinal hormone secretion along with gastrointestinal motility, enhancing the intestinal epithelial barrier, putatively modulating GM composition, and exerting anti-inflammatory, anti-oxidative or toxic effects on systemic circulation (Zhang et al. 2022). As Fang et al. (2022) uncovered, IPA ameliorates sepsis-induced mortality and decreases the serum levels of proinflammatory cytokines by modulating intestinal microbiota, which could provide a new potential therapeutic approach for sepsis. Consistent with the abovementioned research, Huang et al. (2022) also found that the IPA level in the feces of septic patients is significantly lower than in controls, and a lower IPA level was about poorer clinical outcomes. It was also discovered from animal models that there was a relationship between the survival of mice with sepsis and GM-derived IPA, and the treatment with IPA was able to prevent sepsis-related mortality while mitigating the organ injury plus bacterial burden resulting from sepsis.

In addition, we identified *Tyzzarella* on the genus level was negatively connected to sepsis. *Tyzzarella* genus is a member of *Lachnospiraceae* family, which is a potential research highlight. Yutin and Galperin (2013) first named *Tyzzarella* genus by 16S rRNA and

ribosomal protein sequences in 2013. Nonetheless, the detailed functions of *Tyzzarella* have not been elaborated yet. Despite the minimal number of research describing *Tyzzarella* genus, patients at a high risk of cardiovascular diseases manifest an increased level of this genus, which has correlations with a high risk of lifetime cardiovascular diseases as well as a raised cardiovascular disease risk (Murphy et al. 2021). In addition, *Tyzzarella* genus is related to inflammatory bowel disease. *Tyzzarella* genus has been reported to be increased in ulcerative colitis patients from China (Zhu et al. 2022).

Compared with that in patients without Crohn's disease, the abundance of *Tyzzarella* was remarkably increased in patients who have Crohn's disease (Olaisen et al. 2021). Liu et al. (2021) observed that *Tyzzarella* served as a critical species with a notably elevated abundance in SARS-CoV-2 infection, and its AUCs for distinguishing patients with asymptomatic or symptomatic SARS-CoV-2 infection from healthy controls were 70%, and 75%, suggesting that as a bacterial taxonomic biomarker, *Tyzzarella* has potential diagnostic value for SARS-CoV-2 infection.

Moreover, one study showed that a higher intake of saturated fatty acids and trans-fatty acids leads to a prominently raised relative abundance of *Tyzzarella*, which was associated with fatty acid intake (Xu et al. 2022). Pitifully, the exact potential mechanism of *Tyzzarella* in mediating the adverse effects of these diseases is unclear. So far, no research has explored the relationship and mechanism of *Tyzzarella* genus and sepsis, but some studies have elucidated the relationship between *Lachnospiraceae* family and sepsis. Sun et al. (2023) examined the possible function of gut microbiome and metabolites in affecting sepsis severity. They indicated that *Lachnospiraceae* were found to have promising benefits for sepsis progression. There was an intimate correlation between *Lachnospiraceae* species and other sepsis-related metabolites. Overall, further research on the role and mechanism of *Tyzzarella* genus in sepsis is needed.

In general, our study had several limitations. First, while most participants in the GWASs providing summary data for this study had European ancestry, a minority of GM data came from collections acquired through other ethnic groups, possibly leading to racial bias. Secondly, merely the genus level, instead of more specific levels like strain and species, was selected to explore bacterial taxa. More detailed and accurate results may be obtained by analyzing microbiome GWAS via more advanced shotgun metagenomic sequencing. Thirdly, genus was set as the lowest taxonomic level for the data about exposure, and such a limitation prohibited further investigation into the causality between GM and sepsis from the aspect of species.

Conclusions

To sum up, the results of this study provide evidence for underlying causal protective effects of different taxa levels Actinobacteria and *Bifidobacteriaceae*, *Tyzzellerella* genus, and Gastranaerophilales order on sepsis, which provide novel insights into GM functions on sepsis in terms of pathogenesis and treatment.

Ethical statement

For the MR analysis, GM and sepsis summary statistics from GWAS were employed. Each GWAS included in our analysis has been ethically approved by the participating institutions and is publicly accessible through the original research.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Adelman MW, Woodworth MH, Langelier C, Busch LM, Kempker JA, Kraft CS, Martin GS. The gut microbiome's role in the development, maintenance, and outcomes of sepsis. *Crit Care*. 2020 Jun;24(1):278. <https://doi.org/10.1186/s13054-020-02989-1>

Anggraini D, Hasni D, Amelia R. Pathogenesis of sepsis. *Sci J*. 2022; 1(4):332–339. <https://doi.org/10.56260/sciena.v1i4.63>

Cai X, Deng L, Ma X, Guo Y, Feng Z, Liu M, Guan Y, Huang Y, Deng J, Li H, et al. Altered diversity and composition of gut microbiota in Wilson's disease. *Sci Rep*. 2020 Dec;10(1):21825. <https://doi.org/10.1038/s41598-020-78988-7>

Chancharoenthana W, Kamolratanakul S, Schultz MJ, Leela-havanichkul A. The leaky gut and the gut microbiome in sepsis – targets in research and treatment. *Clin Sci (Lond)*. 2023 Apr; 137(8): 645–662. <https://doi.org/10.1042/CS20220777>

Choi SW, O'Reilly PF. PRSice-2: Polygenic Risk Score software for biobank-scale data. *Gigascience*. 2019 Jul;8(7):giz082. <https://doi.org/10.1093/gigascience/giz082>

Di Gioia D, Bozzi Cionci N, Baffoni L, Amoruso A, Pane M, Mogna L, Gaggia F, Lucenti MA, Bersano E, Cantello R, et al. A prospective longitudinal study on the microbiota composition in amyotrophic lateral sclerosis. *BMC Med*. 2020 Jun;18(1):153. <https://doi.org/10.1186/s12916-020-01607-9>

Di Rienzi SC, Sharon I, Wrighton KC, Koren O, Hug LA, Thomas BC, Goodrich JK, Bell JT, Spector TD, Banfield JF, et al. The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. *Elife*. 2013 Oct;2:e01102. <https://doi.org/10.7554/eLife.01102>

Durazzi F, Sala C, Castellani G, Manfreda G, Remondini D, De Cesare A. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Sci Rep*. 2021 Feb;11(1):3030. <https://doi.org/10.1038/s41598-021-82726-y>

Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, Machado FR, Mcintyre L, Ostermann M, Prescott HC, et al. Executive summary: Surviving Sepsis Campaign: International Guidelines for the Management of Sepsis and Septic Shock 2021. *Crit Care Med*. 2021 Nov;49(11):1974–1982. <https://doi.org/10.1097/CCM.0000000000005357>

Fang H, Fang M, Wang Y, Zhang H, Li J, Chen J, Wu Q, He L, Xu J, Deng J, et al. Indole-3-propionic acid as a potential therapeutic agent for sepsis-induced gut microbiota disturbance. *Microbiol Spectr*. 2022 Jun;10(3):e0012522. <https://doi.org/10.1128/spectrum.00125-22>

Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, et al. The MR-BASE platform supports systematic causal inference across the human phenome. *Elife*. 2018 May;7:e34408. <https://doi.org/10.7554/eLife.34408>

Hu C, Rzymiski P. Non-photosynthetic Melainabacteria (Cyanobacteria) in human gut: Characteristics and association with health. *Life*. 2022 Mar;12(4):476. <https://doi.org/10.3390/life12040476>

Huang ZB, Hu Z, Lu CX, Luo SD, Chen Y, Zhou ZP, Hu JJ, Zhang FL, Deng F, Liu KX. Gut microbiota-derived indole 3-propionic acid partially activates aryl hydrocarbon receptor to promote macrophage phagocytosis and attenuate septic injury. *Front Cell Infect Microbiol*. 2022 Oct;12:1015386. <https://doi.org/10.3389/fcimb.2022.1015386>

Kang H, Thomas RM. Bacteria and sepsis: Microbiome to the rescue? *J Clin Med*. 2021 Aug;10(16):3578. <https://doi.org/10.3390/jcm10163578>

Klingensmith NJ, Coopersmith CM. Gut microbiome in sepsis. *Surg Infect*. 2023 Apr;24(3):250–257. <https://doi.org/10.1089/sur.2022.420>

Kullberg RFJ, Wiersinga WJ, Haak BW. Gut microbiota and sepsis: from pathogenesis to novel treatments. *Curr Opin Gastroenterol*. 2021 Nov;37(6):578–585. <https://doi.org/10.1097/MOG.0000000000000781>

Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, Le Roy CI, Raygoza Garay JA, Finnicum CT, Liu X, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat Genet*. 2021 Feb;53(2):156–165. <https://doi.org/10.1038/s41588-020-00763-1>

Kurki MJ, Karjalainen J, Paltia P, Sipilä TP, Kristiansson K, Donner KM, Reeve MP, Laivuori H, Aavikko M, Kaunisto MA, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023 Jan;613(7944):508–518. <https://doi.org/10.1038/s41586-022-05473-8>

Liu Y, Zhang H, Tang X, Jiang X, Yan X, Liu X, Gong J, Mew K, Sun H, Chen X, et al. Distinct metagenomic signatures in the SARS-CoV-2 infection. *Front Cell Infect Microbiol*. 2021 Dec;11:706970. <https://doi.org/10.3389/fcimb.2021.706970>

Longhitano Y, Zanza C, Thangathurai D, Taurone S, Kozel D, Racca F, Audo A, Ravera E, Migneco A, Piccioni A, et al. Gut alterations in septic patients: A biochemical literature review. *Rev Recent Clin Trials*. 2020;15(4):289–297. <https://doi.org/10.2174/1574887115666200811105251>

Mancino W, Lugli GA, Sinderen DV, Ventura M, Turrone F. Mobilome and resistome reconstruction from genomes belonging to members of the *Bifidobacterium* genus. *Microorganisms*. 2019 Dec; 7(12):638. <https://doi.org/10.3390/microorganisms7120638>

Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: A systematic review and meta-analysis. *Crit Care*. 2016 Aug;19:262. <https://doi.org/10.1186/s13054-016-1434-y>

Mbatchou J, Barnard L, Backman J, Marcketta A, Kosmicki JA, Ziyatdinov A, Benner C, O'Dushlaine C, Barber M, Boutkov B, et al. Computationally efficient whole-genome regression for quantitative and binary traits. *Nat Genet*. 2021 Jul;53(7):1097–1103. <https://doi.org/10.1038/s41588-021-00870-7>

Miller WD, Keskey R, Alverdy JC. Sepsis and the microbiome: A vicious cycle. *J Infect Dis*. 2021 Jun;223(Suppl_3):S264–S269. <https://doi.org/10.1093/infdis/jiaa682>

- Murphy K, O'Donovan AN, Caplice NM, Ross RP, Stanton C. Exploring the gut microbiota and cardiovascular disease. *Metabolites*. 2021 Jul;11(8):493. <https://doi.org/10.3390/metabo11080493>
- Niu M, Chen P. Crosstalk between gut microbiota and sepsis. *Burns Trauma*. 2021 Oct;9:tkab036. <https://doi.org/10.1093/burnst/tkab036>
- Olaissen M, Flatberg A, Granlund AVB, Royset ES, Martinsen TC, Sandvik AK, Fossmark R. Bacterial mucosa-associated microbiome in inflamed and proximal noninflamed ileum of patients with Crohn's disease. *Inflamm Bowel Dis*. 2021 Jan;27(1):12–24. <https://doi.org/10.1093/ibd/izaa107>
- Papadimitriou N, Dimou N, Tsilidis KK, Banbury B, Martin RM, Lewis SJ, Kazmi N, Robinson TM, Albanes D, Aleksandrova K, et al. Physical activity and risks of breast and colorectal cancer: A Mendelian randomisation analysis. *Nat Commun*. 2020 Jan;11(1):597. <https://doi.org/10.1038/s41467-020-14389-8>
- Peng Y, Wei J, Jia X, Luan F, Man M, Ma X, Luo Y, Li Y, Li N, Wang Q, et al. Changes in the microbiota in different intestinal segments of mice with sepsis. *Front Cell Infect Microbiol*. 2023 Jan;12:954347. <https://doi.org/10.3389/fcimb.2022.954347>
- Prevel R, Enaud R, Orioux A, Camino A, Berger P, Boyer A, Delhaes L, Gruson D. Gut bacteriobiota and mycobiota are both associated with Day-28 mortality among critically ill patients. *Crit Care*. 2022 Apr;26(1):105. <https://doi.org/10.1186/s13054-022-03980-8>
- R Core Team. A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2020 [cited 2023 Oct 10]. Available from <https://www.r-project.org>
- Robertson C, Savva GM, Clapuci R, Jones J, Maimouni H, Brown E, Minocha A, Hall LJ, Clarke P. Incidence of necrotising enterocolitis before and after introducing routine prophylactic *Lactobacillus* and *Bifidobacterium* probiotics. *Arch Dis Child Fetal Neonatal Ed*. 2020 Jul;105(4):380–386. <https://doi.org/10.1136/archdischild-2019-317346>
- Rosario D, Bidkhorji G, Lee S, Bedarf J, Hildebrand F, Le Chatelier E, Uhlen M, Ehrlich SD, Proctor G, Wüllner U, et al. Systematic analysis of gut microbiome reveals the role of bacterial folate and homocysteine metabolism in Parkinson's disease. *Cell Rep*. 2021 Mar;34(9):108807. <https://doi.org/10.1016/j.celrep.2021.108807>
- Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, VanderWeele TJ, Higgins JPT, Timpson NJ, Dimou N, et al. Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian randomization: The STROBE-MR Statement. *JAMA*. 2021 Oct;326(16):1614–1621. <https://doi.org/10.1001/jama.2021.18236>
- Sun S, Wang D, Dong D, Xu L, Xie M, Wang Y, Ni T, Jiang W, Zhu X, Ning N, et al. Altered intestinal microbiome and metabolome correspond to the clinical outcome of sepsis. *Crit Care*. 2023 Mar;27(1):127. <https://doi.org/10.1186/s13054-023-04412-x>
- Ventura M, Turrioni F, Lugli GA, van Sinderen D. Bifidobacteria and humans: Our special friends, from ecological to genomics perspectives. *J Sci Food Agric*. 2014 Jan;94(2):163–168. <https://doi.org/10.1002/jsfa.6356>
- Wei R, Chen X, Hu L, He Z, Ouyang X, Liang S, Dai S, Sha W, Chen C. Dysbiosis of intestinal microbiota in critically ill patients and risk of in-hospital mortality. *Am J Transl Res*. 2021 Mar;13(3):1548–1557.
- Xiong L, Li Y, Li J, Yang J, Shang L, He X, Liu L, Luo Y, Xie X. Intestinal microbiota profiles in infants with acute gastroenteritis caused by rotavirus and norovirus infection: A prospective cohort study. *Int J Infect Dis*. 2021 Oct;111:76–84. <https://doi.org/10.1016/j.ijid.2021.08.024>
- Xu AA, Kennedy LK, Hoffman K, White DL, Kanwal F, El-Serag HB, Petrosino JF, Jiao L. Dietary fatty acid intake and the colonic gut microbiota in humans. *Nutrients*. 2022 Jun;14(13):2722. <https://doi.org/10.3390/nu14132722>
- Yutin N, Galperin MY. A genomic update on clostridial phylogeny: Gram-negative spore formers and other misplaced clostridia. *Environ Microbiol*. 2013 Oct;15(10):2631–2641. <https://doi.org/10.1111/1462-2920.12173>
- Zhang B, Jiang M, Zhao J, Song Y, Du W, Shi J. The mechanism underlying the influence of indole-3-propionic acid: A relevance to metabolic disorders. *Front Endocrinol (Lausanne)*. 2022 Mar;13:841703. <https://doi.org/10.3389/fendo.2022.841703>
- Zhang W, Sun Z, Zhang Q, Sun Z, Su Y, Song J, Wang B, Gao R. Preliminary evidence for an influence of exposure to polycyclic aromatic hydrocarbons on the composition of the gut microbiota and neurodevelopment in three-year-old healthy children. *BMC Pediatr*. 2021 Feb;21(1):86. <https://doi.org/10.1186/s12887-021-02539-w>
- Zhu L, Xu F, Wan W, Yu B, Tang L, Yang Y, Du Y, Chen Z, Xu H. Gut microbial characteristics of adult patients with allergy rhinitis. *Microb Cell Fact*. 2020 Sep;19(1):171. <https://doi.org/10.1186/s12934-020-01430-0>
- Zhu S, Han M, Liu S, Fan L, Shi H, Li P. Composition and diverse differences of intestinal microbiota in ulcerative colitis patients. *Front Cell Infect Microbiol*. 2022 Aug;12:953962. <https://doi.org/10.3389/fcimb.2022.953962>

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