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Original Article

Gut Microbiome Signatures Predict ICU Delirium Risk in Mechanically Ventilated Older Adults

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ABSTRACT

Background: ICU delirium is a frequent neurological complication of critical illness, while emerging evidence suggests that intestinal dysbiosis may contribute to neurological vulnerability through microbiota-gut-brain interactions. Objective: To investigate the relationship between early gut microbiome characteristics and ICU delirium in mechanically ventilated adults aged ≥65 years. Methods: This analytical observational study included 85 mechanically ventilated older adults treated in tertiary-care ICUs in Punjab between September and December 2025. Fecal samples obtained within 48 hours of mechanical ventilation were analysed using 16S rRNA gene sequencing. Delirium was assessed twice daily using CAM-ICU. Microbial diversity and selected bacterial families were compared according to delirium status, followed by multivariable logistic regression adjusting for age, APACHE II score, and broad-spectrum antibiotic exposure. Results: Delirium occurred in 38/85 participants (44.7%). Shannon diversity was lower in participants with delirium than without delirium (2.14±0.45 vs 3.12±0.58; mean difference −0.98, 95% CI −1.20 to −0.76; p<0.001). Lachnospiraceae (4.2% vs 12.8%) and Ruminococcaceae (3.6% vs 9.4%) were depleted, whereas Enterococcaceae was increased (8.9% vs 2.4%). Higher Shannon diversity (aOR 0.35, 95% CI 0.18–0.68; p=0.003) and greater Lachnospiraceae abundance (aOR 0.78, 95% CI 0.64–0.94; p=0.011) were independently associated with lower odds of delirium. Conclusion: ICU delirium was associated with reduced microbial diversity and altered bacterial composition. These findings support further prospective validation of gut microbiome characteristics as potential markers of delirium vulnerability. Keywords: Acute Disease; Biomarkers; Delirium; Dysbiosis; Gastrointestinal Microbiome; Geriatrics; Respiration, Artificial.

INTRODUCTION

Delirium is a common and clinically consequential neuropsychiatric complication of critical illness, particularly among older adults requiring invasive mechanical ventilation. It is characterized by an acute and fluctuating disturbance in attention, awareness, and cognition and is associated with prolonged mechanical ventilation, longer intensive care unit (ICU) and hospital stays, greater healthcare resource utilization, persistent cognitive impairment, and increased mortality (1,2). Mechanically ventilated older adults are especially vulnerable because advanced age, severe acute illness, physiological instability, medication exposure, and pre-existing reductions in neurological reserve frequently coexist. Although several clinical risk factors for ICU delirium have been identified, their ability to distinguish which individual patients will develop delirium remains limited, creating a need for biologically informative markers that could improve early risk characterization.

Current concepts of delirium pathophysiology implicate interactions among systemic inflammation, endothelial dysfunction, blood-brain barrier disturbance, altered cerebral physiology, and neuroinflammation (1,3). Critical illness can produce a pronounced systemic inflammatory response in which circulating inflammatory mediators influence cerebral endothelial integrity and neuronal homeostasis. These processes may disrupt neurotransmitter balance and neuronal network function, contributing to the acute fluctuations in cognition and attention that characterize delirium. Age-associated vulnerability, pre-existing cognitive impairment, severity of acute illness, infection, and exposure to sedative or other psychoactive medications may further modify this response. Nevertheless, delirium is increasingly understood as a multifactorial syndrome rather than the consequence of a single neurological or inflammatory pathway (1,3).

Growing evidence has expanded this framework to include the microbiota-gut-brain axis as a potential contributor to neurological dysfunction during critical illness. The intestinal microbiome represents a complex microbial ecosystem that participates in immune regulation, epithelial barrier maintenance, nutrient metabolism, and the production of biologically active metabolites capable of interacting with systemic and neural pathways (4,5). A diverse intestinal microbial community contributes to intestinal homeostasis, whereas critical illness can rapidly disturb this ecosystem. Broad-spectrum antimicrobial therapy, altered nutritional intake, physiological stress, severe infection, vasoactive treatment, and other ICU-related exposures may contribute to loss of microbial diversity and expansion of potentially pathogenic organisms (5). Such dysbiosis may coincide with disruption of intestinal barrier integrity and altered production of microbial metabolites, providing a biologically plausible pathway linking intestinal disturbances with systemic inflammatory and neurological processes.

The possible relevance of this relationship to delirium has received increasing attention. Dysbiosis has been implicated in several neurological and neurocognitive disorders, and emerging clinical evidence indicates that gut microbial composition may also differ in acutely ill older adults with delirium (4,6). In particular, reduced microbial diversity and depletion of bacterial taxa involved in short-chain fatty acid production may be relevant because these organisms participate in intestinal metabolic and immune homeostasis. Conversely, expansion of opportunistic or pro-inflammatory taxa may reflect a disturbed intestinal ecosystem occurring alongside severe systemic illness. However, evidence specifically examining early gut microbiome characteristics in mechanically ventilated older adults in relation to ICU delirium remains limited. This uncertainty is clinically important because the microbiome can be assessed through non-invasive biological sampling and may provide information complementary to conventional demographic and illness-severity measures.

A clearer understanding of microbiome patterns associated with delirium could therefore help define biological phenotypes of neurological vulnerability during critical illness and provide a foundation for subsequent development of microbiome-informed risk models. Such associations must, however, be distinguished from demonstrated clinical prediction or causation until temporal relationships, model performance, and external validity have been established. Accordingly, this study investigated the relationship between early gut microbiome composition and ICU delirium among mechanically ventilated adults aged 65 years or older, with particular emphasis on microbial alpha diversity and the relative abundance of selected bacterial families. The study further evaluated whether these microbial characteristics remained associated with delirium after adjustment for age, severity of illness, and broad-spectrum antibiotic exposure.

MATERIALS AND METHODS

This analytical observational study was conducted in the intensive care units of tertiary care referral hospitals in the Industrial and Urban Core Punjab region between September and December 2025. The investigation evaluated the relationship between gut microbiome characteristics measured early after initiation of invasive mechanical ventilation and ICU delirium status assessed through repeated

standardized clinical evaluations. The geographical setting comprised a densely populated and highly industrialized region of Punjab with a substantial clinical burden of severe metabolic and respiratory illnesses requiring tertiary-level critical care.

A total analytical sample of 85 participants was targeted and achieved. The cohort size was informed by previous critical-care pilot investigations of acute gut dysbiosis in respiratory failure in which highly characterized cohorts of approximately 70–90 patients were sufficient to identify substantial differences in microbial alpha diversity and taxonomic abundance. Eligible participants were adults aged 65 years or older who required invasive mechanical ventilation within 24 hours of admission to the ICU. Patients were excluded if they had a pre-existing diagnosis of dementia, active gastrointestinal malignancy, a history of major bowel resection, recent treatment for Clostridioides difficile infection, or a history of severe neuropsychiatric disorders that could interfere with the assessment and interpretation of acute cognitive dysfunction.

Fecal material was obtained within the first 48 hours after initiation of mechanical ventilation using rectal swabs or stool collection bags. Samples were immediately stored at −80°C to preserve microbial material before laboratory analysis. Gut microbial composition was characterized by 16S ribosomal RNA gene sequencing, from which microbial diversity indices and relative taxonomic abundances were determined. The principal microbiome measures included alpha-diversity indices and the relative abundance of bacterial families of clinical and biological interest, including Lachnospiraceae, Ruminococcaceae, Enterococcaceae, and Bacteroidaceae. These microbial measures constituted the principal exposures evaluated in relation to ICU delirium.

Clinical and demographic information was extracted from electronic medical records concurrently with microbiome assessment. Recorded variables included age, sex, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, exposure to broad-spectrum antibiotics during the ICU stay, and duration of mechanical ventilation. APACHE II scores were used to represent baseline severity of critical illness, while age and broad-spectrum antibiotic exposure were considered potential clinical confounders in the adjusted analysis because of their potential relationships with both microbiome composition and delirium occurrence.

The primary clinical outcome was ICU delirium. Participants underwent twice-daily assessment by trained critical-care research nurses using the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU). The CAM-ICU was developed and validated for delirium assessment in critically ill patients, including individuals unable to communicate verbally because of mechanical ventilation (7). The instrument evaluates the characteristic domains of acute or fluctuating change in mental status, inattention, altered level of consciousness, and disorganized thinking and provides a standardized approach to identifying delirium in mechanically ventilated ICU populations. Repeated assessments were used to classify participants according to the occurrence of ICU delirium during the clinical observation period.

Several measures were incorporated to limit clinical heterogeneity and confounding. Restriction to patients aged at least 65 years requiring invasive mechanical ventilation provided a clinically defined target population, while exclusion of patients with dementia, severe neuropsychiatric disorders, major structural gastrointestinal alterations, gastrointestinal malignancy, and recent C. difficile treatment reduced important sources of cognitive and microbiome-related heterogeneity. Delirium was assessed repeatedly using the same standardized clinical instrument by trained critical-care research nurses, and adjusted analyses incorporated age, APACHE II score, and broad-spectrum antibiotic exposure as prespecified clinical covariates.

Continuous clinical and microbiome variables were assessed for distributional normality using the Shapiro-Wilk test before parametric comparison. Between-group differences in continuous variables, including microbial alpha-diversity indices and targeted bacterial relative abundances, were evaluated

using independent-samples t tests. Categorical characteristics were compared between participants with and without ICU delirium using the Chi-square test. Multivariable logistic regression was performed to examine whether gut microbiome characteristics remained independently associated with ICU delirium after adjustment for potential clinical confounding. The adjusted model incorporated chronological age, baseline APACHE II score, and exposure to broad-spectrum antibiotics during the ICU stay together with the microbiome variables evaluated as independent correlates of delirium. Adjusted odds ratios with 95% confidence intervals and corresponding p values were used to quantify these associations.

Ethical approval was obtained from the institutional Ethics Review Committee before initiation of the study. Because participants were critically ill, mechanically ventilated, and could have impaired decision-making capacity related to sedation or acute cognitive disturbance, written informed consent was obtained from their legally authorized representatives before participation and biological sample collection.

RESULTS

Of 95 eligible patients screened in the participating intensive care units, 10 were excluded because of missing baseline fecal samples or unexpected early transfer, leaving 85 mechanically ventilated adults aged 65 years or older in the final analysis. During the observation period, 38 participants (44.7%) were classified as having ICU delirium on repeated CAM-ICU assessment, whereas 47 (55.3%) remained delirium-free. The overall mean age was 71.4 ± 5.1 years, 46 participants (54.1%) were male, and the mean APACHE II score was 22.8 ± 4.3. Broad-spectrum antibiotic exposure was recorded in 72 participants (84.7%).

As shown in Table 1, age, sex, APACHE II score, and antibiotic exposure did not differ statistically between participants with and without delirium. Mean age was 72.1 ± 4.9 years in the delirium group and 70.8 ± 5.2 years in the non-delirium group, corresponding to a mean difference of 1.30 years (95% CI −0.89 to 3.49; p=0.245). Mean APACHE II scores were 23.4 ± 4.1 and 22.3 ± 4.4, respectively, with a mean difference of 1.10 points (95% CI −0.74 to 2.94; p=0.248). In contrast, duration of mechanical ventilation, a post-baseline clinical-course measure, was greater among participants with delirium (7.9 ± 2.2 days) than among those without delirium (5.9 ± 2.1 days), yielding a mean difference of 2.00 days (95% CI 1.06 to 2.94; p<0.001) and a large standardized difference (Hedges g=0.92).

Table 1. Demographic and Clinical Characteristics of Participants (N=85)

Variable	Total Sample (N=85)	Delirium Group (n=38)	Non-Delirium Group (n=47)	Mean Difference (95% CI)	p-value
Age, years	71.4 ± 5.1	72.1 ± 4.9	70.8 ± 5.2	1.30 (−0.89 to 3.49)	0.245
Male sex, n (%)	46 (54.1)	22 (57.9)	24 (51.1)	—	0.531
APACHE II score	22.8 ± 4.3	23.4 ± 4.1	22.3 ± 4.4	1.10 (−0.74 to 2.94)	0.248
Broad-spectrum antibiotic exposure, n (%)	72 (84.7)	34 (89.5)	38 (80.9)	—	0.278
Duration of mechanical ventilation, days	6.8 ± 2.4	7.9 ± 2.2	5.9 ± 2.1	2.00 (1.06 to 2.94)	<0.001

Data are presented as mean ± SD unless otherwise indicated. Mean differences are calculated as delirium minus non-delirium values. Continuous variables were compared using independent-samples tests and categorical variables using Chi-square tests. Duration of mechanical ventilation represents a post-baseline clinical-course measure rather than a baseline characteristic.

Microbial alpha diversity differed substantially between the two groups (Table 2). The mean Shannon Diversity Index was 2.14 ± 0.45 among participants with delirium compared with 3.12 ± 0.58 among those without delirium, corresponding to a mean difference of −0.98 (95% CI −1.20 to −0.76; p<0.001). The standardized difference was large (Hedges g=−1.85). A similar pattern was observed for the Simpson Index, which was lower in the delirium group by 0.16 units (95% CI −0.21 to −0.11; p<0.001; Hedges g=−1.52). Chao1 richness was also lower among participants with delirium, with means of 142.5 ± 32.4 and 215.8 ± 41.6 in the delirium and non-delirium groups, respectively. The corresponding mean difference was −73.3 (95% CI −89.27 to −57.33; p<0.001), with a Hedges g of −1.92.

Table 2. Comparison of Gut Microbiome Alpha-Diversity Measures

Diversity Measure	Delirium Group (n=38)	Non-Delirium Group (n=47)	Mean Difference (95% CI)	Hedges g	p-value
Shannon Diversity Index	2.14 ± 0.45	3.12 ± 0.58	−0.98 (−1.20 to −0.76)	−1.85	<0.001
Simpson Index (1−D)	0.68 ± 0.12	0.84 ± 0.09	−0.16 (−0.21 to −0.11)	−1.52	<0.001
Chao1 richness	142.5 ± 32.4	215.8 ± 41.6	−73.30 (−89.27 to −57.33)	−1.92	<0.001

Values are mean ± SD. Mean differences are calculated as delirium minus non-delirium values. Ninety-five percent confidence intervals and Hedges g were calculated from the reported group means, SDs, and sample sizes. Negative effect sizes indicate lower values in the delirium group.

Differences were also observed in the relative abundance of several targeted bacterial families (Table 3). Lachnospiraceae abundance averaged 4.2 ± 1.8% in participants with delirium compared with 12.8 ± 3.5% in those without delirium, a difference of −8.6 percentage points (95% CI −9.77 to −7.43; p<0.001). Ruminococcaceae abundance was similarly lower in the delirium group (3.6 ± 1.5% versus 9.4 ± 2.8%), corresponding to a mean difference of −5.8 percentage points (95% CI −6.75 to −4.85; p<0.001). Both differences were large when standardized, with Hedges g values of −2.97 and −2.49, respectively. Conversely, Enterococcaceae abundance was higher among participants with delirium, with mean relative abundances of 8.9 ± 3.1% and 2.4 ± 1.1% in the delirium and non-delirium groups, respectively. The resulting difference was 6.5 percentage points (95% CI 5.44 to 7.56; p=0.002), with a Hedges g of 2.89. Bacteroidaceae abundance showed a comparatively small between-group difference: 14.1 ± 4.6% in participants with delirium and 15.5 ± 5.1% in those without delirium. The mean difference was −1.4 percentage points (95% CI −3.50 to 0.70; p=0.204; Hedges g=−0.28).

Table 3. Relative Abundance of Targeted Bacterial Families According to ICU Delirium Status

Bacterial Family	Delirium Group (n=38)	Non-Delirium Group (n=47)	Mean Difference, percentage points (95% CI)	Hedges g	p-value
Lachnospiraceae	4.2 ± 1.8%	12.8 ± 3.5%	−8.60 (−9.77 to −7.43)	−2.97	<0.001
Ruminococcaceae	3.6 ± 1.5%	9.4 ± 2.8%	−5.80 (−6.75 to −4.85)	−2.49	<0.001
Enterococcaceae	8.9 ± 3.1%	2.4 ± 1.1%	6.50 (5.44 to 7.56)	2.89	0.002
Bacteroidaceae	14.1 ± 4.6%	15.5 ± 5.1%	−1.40 (−3.50 to 0.70)	−0.28	0.204

Values represent relative abundance as mean ± SD. Mean differences are calculated as delirium minus non-delirium values. Ninety-five percent confidence intervals and Hedges g were calculated from the reported group means, SDs, and sample sizes. Positive effect sizes indicate greater abundance in the delirium group and negative values indicate lower abundance.

In multivariable logistic regression, Shannon diversity and Lachnospiraceae abundance remained inversely associated with ICU delirium after adjustment for chronological age, APACHE II score, and broad-spectrum antibiotic exposure (Table 4). A higher Shannon Diversity Index was associated with lower adjusted odds of delirium (aOR 0.35, 95% CI 0.18–0.68; p=0.003). Lachnospiraceae abundance was also inversely associated with delirium status (aOR 0.78, 95% CI 0.64–0.94; p=0.011). In contrast, APACHE II score (aOR 1.06, 95% CI 0.92–1.22; p=0.412), age (aOR 1.02, 95% CI 0.91–1.15; p=0.714), and broad-spectrum antibiotic exposure (aOR 1.45, 95% CI 0.52–4.05; p=0.478) were not statistically associated with delirium in the adjusted model.

Table 4. Multivariable Logistic Regression of Factors Associated with ICU Delirium

Variable	Odds Ratio	95% CI	p-value
Shannon Diversity Index	0.35	0.18–0.68	0.003
Lachnospiraceae abundance	0.78	0.64–0.94	0.011
APACHE II score	1.06	0.92–1.22	0.412
Age	1.02	0.91–1.15	0.714
Broad-spectrum antibiotic exposure	1.45	0.52–4.05	0.478

Overall, the unadjusted comparisons showed substantially lower microbial alpha diversity and lower relative abundances of Lachnospiraceae and Ruminococcaceae among participants with ICU delirium, together with higher Enterococcaceae abundance. After adjustment for the reported clinical covariates, Shannon diversity and Lachnospiraceae abundance remained independently associated with delirium

status. These adjusted associations identify microbiome characteristics associated with the observed delirium phenotype but do not, in the absence of reported model discrimination, calibration, or validation analyses, quantify the clinical predictive performance of a microbiome-based risk model.

DISCUSSION

The present study identified distinct differences in gut microbiome composition between mechanically ventilated older adults with and without ICU delirium. Participants with delirium demonstrated consistently lower microbial alpha diversity, including lower Shannon, Simpson, and Chao1 indices, together with marked depletion of Lachnospiraceae and Ruminococcaceae and greater relative abundance of Enterococcaceae. The between-group differences were substantial, particularly for Shannon diversity, Lachnospiraceae, Ruminococcaceae, and Enterococcaceae. After adjustment for age, APACHE II score, and broad-spectrum antibiotic exposure, higher Shannon diversity and greater Lachnospiraceae abundance remained inversely associated with delirium. These findings indicate that altered intestinal microbial ecology accompanies delirium in this critically ill population and support further investigation of the gut microbiome as a biological correlate of acute brain dysfunction.

The association between reduced microbial diversity and delirium is consistent with emerging clinical evidence in acutely ill older populations. Garcez et al. examined gut microbiota in adults aged 65 years or older admitted with acute illness and similarly found that greater alpha diversity was associated with lower odds of delirium after adjustment for clinical confounders (6). That study also identified differences in taxonomic composition between patients with and without delirium, providing direct clinical support for an association between intestinal microbial ecology and acute cognitive dysfunction. The present findings extend this pattern to a particularly vulnerable subgroup of older adults requiring invasive mechanical ventilation, among whom physiological stress, antimicrobial exposure, altered nutrition, and severe systemic illness may exert additional pressure on intestinal microbial homeostasis.

The observed depletion of Lachnospiraceae and Ruminococcaceae is biologically relevant because members of these bacterial groups include important producers of short-chain fatty acids, particularly butyrate. Short-chain fatty acids participate in maintenance of epithelial barrier integrity, regulation of immune activity, and metabolic communication within the microbiota-gut-brain axis (4,5). Experimental evidence further indicates that disruption of short-chain fatty acid metabolism can accompany cognitive and neuroinflammatory abnormalities and that manipulation of gut microbial composition can modify these pathways, although such evidence does not establish equivalent causality in critically ill humans (9). Therefore, the marked depletion of putatively beneficial bacterial families observed in the delirium group provides a plausible mechanistic hypothesis but should not be interpreted as evidence that depletion of these organisms directly caused delirium.

The greater abundance of Enterococcaceae among participants with delirium may similarly represent a disturbed intestinal ecosystem rather than an isolated pathogenic mechanism. Critical illness can rapidly alter the gut environment through antimicrobial therapy, nutritional disruption, altered intestinal motility, impaired mucosal perfusion, systemic inflammation, and exposure to multiple ICU medications (5). These conditions can favor loss of microbial diversity and expansion of opportunistic organisms. The present Enterococcaceae finding therefore complements the overall pattern of dysbiosis rather than demonstrating an organism-specific causal pathway. Importantly, the study did not measure intestinal permeability, circulating endotoxin, inflammatory cytokines, microbial metabolites, or central nervous system inflammatory markers; consequently, proposed biological connections between dysbiosis, systemic inflammation, and delirium remain mechanistic interpretations rather than directly demonstrated pathways.

The findings are also compatible with broader observations linking altered gut microbiota with cognitive and neurodegenerative disorders. In patients with Alzheimer disease, reduced microbial diversity and depletion of butyrate-producing organisms have been described alongside alterations in

microbial functional pathways (8). Related work concerning perioperative neurocognitive dysfunction has proposed that intestinal microbial disturbances may interact with systemic inflammation, neuroimmune signaling, and barrier dysfunction (9). Although chronic neurodegenerative disease and acute ICU delirium differ substantially in temporal course and pathophysiology, these observations collectively support the possibility that disturbances of microbiota-gut-brain communication may occur across several forms of neurological vulnerability. Such cross-condition parallels should nevertheless be interpreted cautiously because microbial signatures are highly dependent on population characteristics, diet, medication exposure, geography, underlying disease, sequencing methodology, and clinical setting.

The adjusted analysis provides additional evidence that the observed microbiome differences were not explained entirely by chronological age or baseline APACHE II score. Higher Shannon diversity was associated with lower adjusted odds of delirium, with an aOR of 0.35 (95% CI 0.18–0.68), while Lachnospiraceae abundance remained inversely associated with delirium (aOR 0.78, 95% CI 0.64–0.94). However, these estimates should be interpreted as adjusted associations rather than proof of clinical prediction. Logistic regression alone does not establish the performance of a predictive model. Evaluation of a clinically useful biomarker model would additionally require assessment of discrimination, calibration, internal validation, reproducibility, and subsequently external validation in independent populations. The present findings therefore support the biological and epidemiological relevance of the identified microbiome characteristics but do not establish a microbiome-based test for routine delirium risk stratification.

Participants with delirium also experienced a longer duration of mechanical ventilation, averaging 7.9 days compared with 5.9 days among those without delirium. This difference is clinically notable but should not be interpreted as a baseline determinant because duration of ventilation evolves during the ICU course and may be influenced by delirium, severity of illness, sedation, complications, or other time-dependent factors. Delirium in critically ill patients is associated with complex interactions among systemic inflammation, neurological vulnerability, severity of illness, and treatment-related exposures (1–3). The longer ventilation duration observed in the delirium group is therefore best considered part of the differing clinical course rather than evidence of a temporally established causal relationship.

Several features strengthen the study. The investigation focused on a clinically homogeneous and high-risk population of mechanically ventilated adults aged at least 65 years. Microbiome samples were obtained early after initiation of mechanical ventilation and stored at −80°C, while delirium was assessed repeatedly using CAM-ICU rather than relying on routine clinical diagnosis alone. Adjustment for age, APACHE II score, and broad-spectrum antibiotic exposure also provided an initial assessment of whether the main microbiome associations persisted after consideration of important clinical characteristics. The magnitude and consistency of the alpha-diversity differences across Shannon, Simpson, and Chao1 measures further strengthen the descriptive microbiome signal.

Several limitations must nevertheless be considered. The observational design prevents causal attribution, and the precise temporal relationship between fecal sampling and the first delirium episode is essential when interpreting the findings as potentially antecedent biomarkers. The sample of 85 participants, including 38 delirium events, also limits the complexity and stability of multivariable modeling and restricts detailed subgroup analyses. Only an early microbiome sample was reported, preventing assessment of dynamic microbial changes during critical illness. Stool material was obtained using either rectal swabs or stool collection bags, and differences between collection methods may contribute to measurement heterogeneity. The adjusted model incorporated age, APACHE II score, and broad-spectrum antibiotic exposure but did not account for several other clinically plausible determinants of both delirium and intestinal microbiota, including sedative and opioid exposure, sepsis, nutritional route, vasopressor treatment, admission diagnosis, comorbidity burden, acid-suppressive therapy, and antimicrobial exposure before microbiome sampling. In addition, relative-abundance microbiome data are compositional, and analysis of selected taxa using conventional parametric

approaches may not fully account for the statistical characteristics of sequencing-derived abundance data.

The use of 16S rRNA sequencing provided taxonomic information but does not directly characterize microbial genes, metabolic pathways, or circulating microbial products. Future investigations should therefore incorporate larger prospective multicentre cohorts, clearly establish that microbiome sampling precedes incident delirium, and obtain serial specimens to characterize longitudinal microbial trajectories. Shotgun metagenomic or metatranscriptomic approaches, combined with measurement of short-chain fatty acids, inflammatory mediators, intestinal permeability markers, and relevant clinical exposures, could help determine whether the observed taxonomic patterns correspond to functional alterations in microbiota-gut-brain signaling. External validation would also be required before any microbial signature could be considered for clinical risk prediction. Microbiome-directed interventions such as nutritional strategies, prebiotics, probiotics, or microbiota transplantation remain research questions rather than established delirium-prevention therapies in this population.

Taken together, the present findings contribute to an emerging clinical literature linking intestinal microbial ecology with acute cognitive dysfunction. They are particularly notable because the associations were demonstrated in mechanically ventilated older adults, a population at substantial risk of both delirium and critical-illness-associated gut dysbiosis. The results support further investigation of reduced microbial diversity and depletion of Lachnospiraceae as candidate biological markers of neurological vulnerability, while emphasizing that temporality, causality, predictive performance, and therapeutic modifiability remain to be established.

CONCLUSION

Mechanically ventilated older adults with ICU delirium exhibited substantially lower gut microbial alpha diversity, reduced relative abundances of Lachnospiraceae and Ruminococcaceae, and greater Enterococcaceae abundance compared with participants without delirium. Higher Shannon diversity and greater Lachnospiraceae abundance remained independently associated with lower odds of delirium after adjustment for age, APACHE II score, and broad-spectrum antibiotic exposure. These findings support an association between altered intestinal microbial ecology and ICU delirium and provide a rationale for prospective longitudinal studies evaluating whether early microbiome characteristics can contribute to reproducible delirium risk assessment. The present results establish neither causality nor the clinical predictive performance of a microbiome-based biomarker model.

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