ORIGINAL

Early and sustained *Lactobacillus plantarum* probiotic therapy in critical illness: the randomised, placebo-controlled, restoration of gut microflora in critical illness trial (ROCIT)

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Abstract

Purpose: In adults requiring treatment in an intensive care unit, probiotic therapy using *Lactobacillus plantarum* 299v may reduce nosocomial infection. The aim of this study was to determine whether early and sustained *L. plantarum* 299v therapy administered to adult ICU patients increased days alive and at home.

Methods: A multicentre, parallel group, placebo-controlled, randomised clinical trial was conducted. Adult patients within 48 h of intensive care admission and expected to require intensive care beyond the day after recruitment were eligible to participate. *L plantarum* 299v or placebo were administered immediately after enrolment and continued for 60 days. The primary outcome was days alive and out of hospital to Day 60 ($DAOH_{60}$). Secondary outcomes included nosocomial infections.

Results: The median [interquartile range (IQR)] number of $DAOH_{60}$ in the probiotic ($n = 110$) and placebo group ($n = 108$) was 49.5 (IQR 37.0–53.0) and 49.0 (IQR 43.8–53.0) respectively, between-group difference of 0.0 [95% confidence interval (CI) − 6.10 to 7.1, $P = 0.55$]. Nosocomial infection occurred in 8 (7.3%) and 5 (4.6%) of the probiotic and placebo group participants, respectively, odds ratio 1.62 (95% CI 0.51–5.10), $P = 0.57$. There were no serious, or probiotic-associated adverse events.

Conclusion: Early and sustained untargeted administration of probiotic therapy with *Lactobacillus plantarum* 299v to adult patients admitted to the ICU is safe, but not associated with improved patient outcomes.

Keywords: Intensive care unit, Critical illness, Probiotics, Nosocomial infection

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Introduction

Critical illness requiring treatment in an intensive care unit (ICU) results in rapid and profound alterations to the gastrointestinal microflora [1, 2]. Microbiota depletion and diversity loss are associated with adverse outcomes including prolonged hospital stay, nosocomial infections and increased mortality [2–4]. The reintroduction of commensal bacteria using strain-specific oral probiotic therapy may mitigate these adverse effects [5, 6].

Lactobacillus (L.) plantarum 299 V is a human commensal that survives passage through the gastrointestinal tract, irrespective of gastric acidity [7]. It reduces gastrointestinal bacterial translocation, attenuates systemic inflammation in critically ill patients, and has in vitro antimicrobial activity against a wide range of potentially pathogenic species [8]. In a meta-analysis of 14 randomised trials involving adult critically ill patients, probiotic therapy decreased overall infections, a benefit most apparent in trials of *L. plantarum* [9]. Given that probiotics exhibit strain-specific effects, *L. plantarum*, as a single agent, is a strong candidate intervention to improve clinical outcomes. However, sufficient evidence to inform clinical practice is limited by trial quality and heterogeneity in the timing, dose and duration of therapy, resulting in conflicting guideline recommendations [10, 11].

The multicentre, randomised, restoration of gut microflora in critical illness trial (ROCIT) was designed to test the hypothesis that, compared with placebo, the early and sustained enteral administration of *L. plantarum* 299v probiotic therapy, in adult patients expected to require ongoing treatment in the ICU beyond the day after recruitment, would improve clinical outcomes including increased days alive and out of hospital to Day 60 ($DAOH_{60}$).

Methods

Trial design

The investigator initiated ROCIT study was a parallel group, placebo-controlled, randomised clinical trial conducted in the ICUs of five hospitals in Perth, Western Australia (ANZCTR 12617000783325). The protocol was prospectively approved by the research ethics committee (HREC) of all participating institutions and reported prior to completion of the study (South Metropolitan Health Service Human Research Ethics Committee ref:RGS000004, St John of God Health Care Human Research Ethics Committee ref:1183) [12]. Initial HREC approval had included the provision to enrol participants who lacked capacity to provide informed consent, where prospective consent was able to be obtained from the person responsible. In June 2018, after 83 participants had been enrolled, the study management committee received an updated interpretation of local legislation from the lead HREC. This mandated that

Take-home message

Early and sustained probiotic administration to adult patients requiring treatment in the intensive care is safe but ineffective in improving outcomes or reducing nosocomial infection. Whether more targeted therapy is beneficial remains uncertain.

trial recruitment of the subsequent 138 participants was restricted to patients competent to provide consent prospectively. Approval to analyse and report the participants enrolled prior to this change was granted by the HREC.
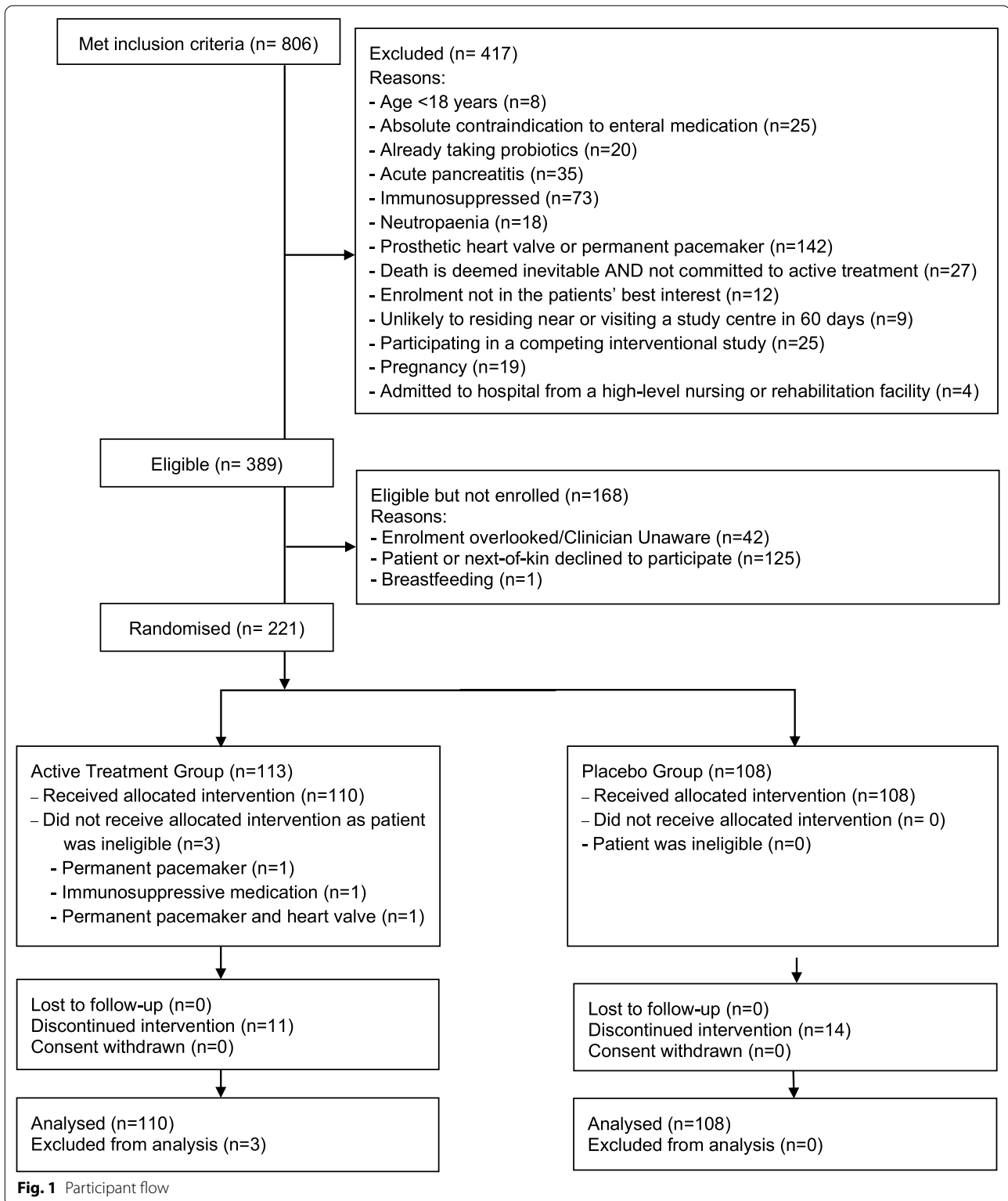
Patients

Eligible patients were adults within 48 h of ICU admission and expected by the treating clinician to require ICU care beyond the calendar day after recruitment. Key exclusion criteria included immunosuppression, presence of a prosthetic heart valve or permanent pacemaker and admission to hospital from a high-level nursing or rehabilitation facility (Fig. 1). The complete exclusion criteria are provided in the Supplementary Appendix (eTable 1).

Randomisation and masking

Variable-block, 1:1 randomisation, stratified by site, was generated using a web-based interface [13]. Allocation concealment was maintained using an unblinded pharmacist to assign unique, sequential numbers to each bottle of study drug. The active study drug and the placebo were prepared by a certified facility in identically packaged capsules with 60 capsules per bottle (Metagenics Australia, 741 Nudgee Road, Northgate, Qld, 4013). All members of the treating team, the study participants, research staff and outcome adjudicators were blinded to the treatment allocation. Unblinding occurred after database lock and completion of the statistical analysis.

Study treatment

The study drug was administered once daily, beginning immediately after enrolment and continued for 60 days. Study participants who were discharged from hospital prior to Day 60 were advised to continue the treatment regime until the course was complete, then return a post-discharge treatment diary and the study drug bottle to the coordinating site. The active study drug contained 2×10^{10} colony-forming units (CFUs) of *L. plantarum* 299v per capsule, a dose comparable or greater than that used in other studies [9]. The placebo was of identical appearance but contained only microcrystalline cellulose. Independent testing of each study drug batch at the lead site confirmed bacterial absence in the placebo capsules and $> 2 \times 10^{10}$ CFUs of *L. plantarum* 299v in the probiotic capsules. Participants were requested to refrain from

Fig. 1 Participant flow

initiating any probiotic treatment during the 60 days of study participation. All other aspects of care were at the discretion of the patient and clinical teams.

Outcome measures

The primary outcome was $DAOH_{60}$. This is a validated composite measure for which the components of death,

index hospital length of stay and the occurrence and duration of hospital readmission, plausibly, may all be improved by probiotic therapy [14]. Days spent in a rehabilitation facility or high-level nursing facility to Day 60 were considered as days in hospital and participants who died prior to Day 60 were recorded as having zero $DAOH_{60}$.

Incident nosocomial infections, a secondary outcome, were assessed independently by two blinded infectious diseases specialist clinicians. These included hospital-acquired pneumonia, ventilator-associated pneumonia, *Clostridioides difficile*-associated diarrhoea, surgical site infection, urinary tract infection, and blood stream infection as defined by Centre for Disease Control (CDC) criteria [15]. Antibiotic-free days were collected for all days in hospital, including any readmissions to Day 60. Quality of life was assessed using the five-level EQ-5D (EQ-5D-5L) questionnaire including the EQ-5D descriptive system with five domains (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) and the EQ visual analogue scale (EQ VAS) [16]. The number of missed study medication days was the sum of the missed days whilst hospitalised, plus, either the number of remaining capsules in returned bottles post-discharge or the number of omitted study diary days post-discharge, whichever was greater.

Statistical analysis

In the placebo group, a mean $DAOH_{60}$ of 37 and standard deviation (SD) of nine was assumed, using previous estimates from the lead study site [12]. Based on a two-sided type I error rate of 0.05 and sample size inflation of 20% to account for rank-based testing, a further 5% each for withdrawn consent and loss to follow-up, a sample size of 220 participants was determined to have 80% power to detect a between-group difference in $DAOH_{60}$ of 4 days. This difference was considered clinically meaningful to ICU consumers [12].

The primary analysis was performed on an intention-to-treat population, defined as all eligible, randomised patients. For non-parametric outcomes, significance was determined using the Wilcoxon rank-sum test, median difference was calculated using quantile regression, with the inversion method used to calculate a 95% confidence interval (CI). Fischer's Exact test and Chi^2 test were used to test association between categorical outcomes as appropriate. Analyses of pre-specified subgroups (sepsis defined according to sepsis-3 criteria [17], antibiotics at enrolment, ICU admission urgency and ICU admission type), and a post hoc septic shock subgroup, included an interaction term between assigned treatment and subgroup using a two-sided hypothesis test. The discrepancy in pre-specified subgroups reported in the trial

registration and published protocol is provided in the Supplementary Appendix. A sensitivity analysis was conducted including all patients receiving study medication for ≥ 80% of days alive to Day 60. Each health state was converted into the corresponding utility index, indicating the preference of being in a health state, with utilities calculated using Australian value weights [18]. Adverse outcomes, based on clinician suspicion of an association with the intervention, were reported for all randomised patients. All serious adverse events, defined and reported according to the Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (CPMP/ICH/377/95), were reported to the Data Safety Management Committee (Membership provided in the Supplementary Appendix). No interim analysis was planned or undertaken. A *P* value of less than 0.05 was deemed statistically significant. No correction was made for multiple comparisons. All data analyses were conducted using R version 3.5.1.

Results

Between July 2017 and December 2019, 221 patients were enrolled in the study (Fig. 1). Three patients were found to be ineligible, leaving an intention-to-treat population of 218. The primary outcome data were available for all participants. Baseline characteristics of the probiotic ($n = 110$) and placebo group ($n = 108$) participants were similar, although 81% of patients in the probiotic group compared with 62% in the placebo group received antibiotics at baseline (Table 1). The number of patients receiving study medication for ≥ 80% of days alive to Day 60 was 95 (86.4%), and 87 (80.6%) in the probiotic and placebo groups, respectively. Other measures of study treatment compliance are provided in the Supplementary Appendix (eTable 2).

Primary outcome

The median [interquartile range (IQR)] number of $DAOH_{60}$ in the probiotic group was 49.5 (IQR 37–53) and 49 (IQR 43.8–53) in the placebo group, with a between-group absolute difference of 0.0 (95% CI − 6.1 to 7.1), $P = 0.55$. There were no significant between-group differences observed in the components of the primary outcome, or when analysis was limited to participants with ≥ 80% compliance (Table 2). There was no significant between-group difference for the four pre-specified subgroup pairs (Table 3).

Secondary outcomes

Nosocomial infection occurred in 8 (7.3%) and 5 (4.6%) of the probiotic and placebo group participants, respectively, odds ratio 1.62 (95% CI 0.51–5.1), $P = 0.57$. No participant had more than one nosocomial infection.

Table 1 Baseline participant characteristics

Characteristic	Probiotics (n = 110)	Placebo (n = 108)
Age—years (SD)	62.1 (15.7)	62.6 (14.5)
Male sex—no. (%)	65 (59.1)	69 (63.9)
APACHE-II score—mean (SD)	14.5 (6)	14.6 (6.9)
APACHE-II Co-morbid conditions—no. (%)		
Respiratory	9 (8.2)	10 (9.3)
Cardiovascular	23 (20.9)	21 (19.4)
Hepatic	3 (2.7)	0 (0)
Renal	7 (6.4)	7 (6.5)
Immunosuppression by disease	0 (0)	0 (0)
Immunosuppression by therapy	0 (0)	0 (0)
Source of admission to ICU—no. (%)		
Emergency department	30 (27.3)	33 (30.6)
Hospital ward	19 (17.3)	23 (21.3)
Transfer from another ICU	1 (0.9)	0 (0)
Transfer from another hospital	17 (15.5)	9 (8.3)
Operating theatre following elective surgery	33 (30)	31 (28.7)
Operating theatre following emergency surgery	10 (9.1)	12 (11.1)
Physiological support—no. (%)		
Vasoactive therapy	67 (60.9)	68 (63)
Mechanical ventilation	18 (16.4)	16 (14.8)
Renal replacement therapy	6 (5.5)	4 (3.7)
Nutritional support—no. (%)		
Normal oral diet	70 (63.6)	61 (56.5)
Enteral nutrition	10 (9.1)	13 (12)
Enteral and parenteral nutrition	0 (0)	1 (0.9)
Parenteral nutrition	1 (0.9)	0 (0)
Fasting	29 (26.4)	33 (30.6)
Pharmacological therapy—no. (%)		
Antibiotic	89 (80.9)	67 (62)
Antifungal	6 (5.5)	6 (5.6)
Antiviral	6 (5.5)	5 (4.6)
Acid suppressive	79 (71.8)	75 (69.4)
Time from ICU admission to randomisation—median days (IQR)	0 (0–1)	0 (0–1)

SD standard deviation, *APACHE* acute physiology and chronic health evaluation, *ICU* intensive care Unit, *IQR* interquartile range

Other clinical outcomes including ICU and hospital mortality were similar between groups (Table 2, eTable 4). Amongst survivors, overall quality of life at Day 60, as assessed by median EQ-5D-5L VAS scores was similar in the probiotic and placebo groups, 75 (IQR 60–85) and 76 (IQR 60–90), respectively, between-group difference − 1.0 (95% CI − 14.5 to 16.3), $P = 0.39$. The individual components scores are provided in the Supplementary Appendix (eTable 5).

A post hoc exploratory analysis suggested significant increase in $DAOH_{60}$ and antibiotic-free days in the sub-group of 24 participants with septic shock at baseline (eTable 3).

Safety

There were no serious adverse events reported amongst the 221 randomised participants, including no cases of Lactobacillus infection. There were three adverse events reported in total, one in the probiotic group and two in the placebo group (eTable 6).

Discussion

In this multicentre, randomised, placebo-controlled clinical trial, the early and sustained administration of probiotic therapy with *L. plantarum* 299v to adult patients admitted to the ICU did not result in a significant difference in days alive and out of hospital to Day

Table 2 Outcomes analysis

Outcome	Probiotics (*n* = 110)	Placebo (*n* = 108)	Median difference (95% CI)	Unadjusted odds ratio (95% CI)	*P* value
Primary outcome					
Days alive and out of hospital to Day 60—median days (IQR)	49.5(37–53)	49(43.8–53)	0 (− 6.1 to 7.1)		0.55
DAOH$_{60}$ components to Day 60					
Mortality —no. (%)	6 (5.45)	5 (4.6)		1.19 (0.4–4)	1.00
Days out of hospital amongst survivors (*n* = 207)—median days (IQR)	50 (40.8–53)	50 (45–3.5)	0 (− 3.4 to 4.9)		0.59
DAOH$_{60}$ amongst participants with ≥ 80% compliance (*n* = 182)—median days (IQR)	49 (36.5–53)	50 (45–54)	− 1 (− 5.1 to 7.1)		0.36
Secondary outcomes					
Nosocomial infection—no. (%)	8 (7.3)	5 (4.6)		1.62 (0.51–5.1)	0.57
Antibiotic-free days—median days (IQR)	53 (48–58)	54 (49–58)	− 1 (− 3.1 to 4.1)		0.46
ICU mortality – no. (%)	4 (3.6)	4 (3.7)		0.98 (0.24–4.03)	1.00
Hospital mortality—no. (%)	5 (4.6)	4 (3.7)		1.24 (0.32–4.74)	1.00
EQ-5D-5L VAS Overall health state (*n* = 195)—median score (IQR)	75 (60–85)	76 (60–90)	− 1.0 (− 14.5 to 16.3)		0.39
EQ-5D-5L Utility index—median (IQR)	0.81 (0.57–1)	0.78 (0.56–1)	0.02 (− 0.07 to 0.07)		0.96

CI confidence interval, *IQR* interquartile range, *DAHO$_{60}$* days alive and out of hospital to Day 60, *EQ-5D-5L* five-level EQ-5D, *VAS* visual analogue scale

Table 3 Subgroup analysis

	Probiotics	Placebo	Median difference (95% CI)	Interaction *P* value
Median DAOH$_{60}$ (IQR)				
Presence or absence of sepsis at enrolment				0.07
Sepsis (*n* = 90)	49.5 (32.5–53)	46.5 (32–50)	2.0 (− 0.9 to 7.9)	
No sepsis (*n* = 128)	49.5 (39.3–53)	51.5 (46–54)	− 1(− 9.7 to 0.7)	
Antibiotics at enrolment				0.52
Receiving antibiotics (*n* = 165)	50 (36.3–53)	49 (41.5–53)	1 (− 5.8 to 6.6)	
Not receiving antibiotics (*n* = 53)	47.5 (37.8–52.3)	51 (46–55)	− 2 (− 11.5–2.5)	
ICU admission urgency				0.58
Elective admission (*n* = 59)	51 (47–52)	53 (47.5–54.5)	− 2 (− 4.4 to 1.2)	
Emergency admission (*n* = 159)	48.5(33.5–53)	47 (39–53)	1 (− 5.2 to 6.4)	
ICU admission type				0.22
Medical admission (*n* = 110)	50 (34–53)	47 (38–53)	3 (− 2.9 to 6.9)	
Surgical admission (*n* = 108)	49 (40.5–52)	51 (46–53)	− 2 (− 5.8 to 2.81)	

CI confidence interval, *IQR* interquartile range, *DAOH$_{60}$* days alive and out of hospital to Day 60

60. Nosocomial infection and all other pre-specified secondary outcomes were also similar between groups. The administration of *L. plantarum* 299v was safe. There were no serious adverse events amongst participants, including no associated Lactobacilli infections, and few reported adverse events.

A systematic review of previous clinical trials suggested a lower incidence of nosocomial infection and improved clinical outcomes amongst critically ill patients receiving probiotic therapy [9]. However, the robust design of the placebo-controlled ROCIT study, powered to detect a meaningful difference in a patient-centred outcome, provides results that are consistent with the findings of other higher quality trials [9]. The ROCIT study extends these findings by evaluating the early administration of a high dose probiotic, sustained amongst survivors

until ascertainment of the primary outcome at 60 days. In addition to a lack of benefit demonstrated amongst the entire cohort, subgroup analyses based on antibiotic administration at time of enrolment, presence or absence of sepsis, ICU admission urgency and type, also failed to demonstrate benefit. Similarly, an analysis of highly compliant participants, though a post-randomisation variable that could not be determined at baseline, did not suggest that the lack of benefit could be explained by insufficient probiotic exposure. Together, these findings suggest that the widespread, untargeted administration of *L. plantarum* 299v to patients admitted to the ICU, although safe, is ineffective.

In a large clinical trial conducted in infants in rural India, the administration of *L. plantarum* decreased the risk of a composite outcome including infection and death [19]. Whilst the microbiota of adults is complex and established, in infants it is newly developing. This may explain why an untargeted approach could be successful in infants but not adults. Untargeted enteral probiotic administration to critically ill patients receiving mechanical ventilation may be of specific benefit due to the risk of aspiration of gastric contents [20]. In addition, the expected mortality of the cohort in this study was relatively low, and mechanically ventilated patients may be expected to have a higher illness severity. Although the attributable mortality of ventilator-associated pneumonia is uncertain, further study of this cohort will provide important information of the role of untargeted probiotics in another high acuity cohort [21].

Alternatively, a more targeted approach, based on a specific gastrointestinal microbiome composition may identify a population of critically ill adults who would benefit from *L. plantarum* administration [22]. Preliminary studies have found an association between gastrointestinal microbiota composition and adverse outcomes from critical illness [23]. To date, however, there is limited evidence that this risk is modifiable [24]. This may be explained by the substantial variation in microbiota disturbance observed between and within individuals after a uniform exposure such as broad spectrum antibiotic [25]. The post hoc analysis suggesting benefit of *L. plantarum* 299v amongst patients with septic shock may be a chance finding and must be considered hypothesis generating, but may be due to consistent, severe dysbiosis in this subgroup. Irrespective, developing targeted interventions will require a mechanistic and individualised understanding of the effects probiotics exert on the gastrointestinal microbiota in critical illness. Developing tools to provide information on the composition of the gastrointestinal microbiota in a clinically relevant timeframe could assist with a goal of precision restoration of gut microbiota constituents and diversity.

There are several limitations to this study. Observed days alive and at home in the placebo group were higher than estimated in the sample size calculation so that the findings may be underpowered. However, on the basis of the minimal between-group differences in all outcome measures, a false-negative finding is considered unlikely. Although the trial was initially approved to include participants who lacked capacity to provide consent, the approval to enrol incapacitated patients was rescinded after approximately one third of the participants were enrolled, and enrolment then limited to participants with capacity to provide prospective consent. Incapacitated patients may differ in important characteristics, including illness severity, and overall mortality of 5% is low for an ICU cohort. However, outcomes were similar amongst planned and unplanned ICU admissions, suggesting that identifying a cohort based on higher acuity alone is unlikely to lead to different findings. Reliance on clinical suspicion of an adverse event may increase the chance of underreporting, although lack of any outcome difference suggests that any such effect resulted in minimal impact. Finally, whether *L. plantarum* 299v administration targeted to a subgroup of critically ill patients, or whether untargeted administration of an alternative probiotic is beneficial, remains uncertain.

Conclusion

The early and sustained administration of probiotic therapy with *L. plantarum* 299v to adult patients admitted to the ICU did not result in a significant difference in days alive and at home to Day 60.

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Author contributions
EL: conceived the study, wrote the first draft of the protocol, chaired the study management committee, supervised the data analysis, wrote the first draft of the manuscript and takes overall responsibility for the study. MA, AC, AC, JG, JL, AP, AR, BR, SS, TS and BW: contributed to the study design, protocol development, data analysis and redrafting the study manuscript. DB contributed to the study design, protocol development, and redrafting of the study manuscript. JF, A-MP and SW: contributed to the study design, protocol development, data collection and redrafting the study manuscript. AH: contributed to the study design, protocol development, data analysis and redrafting the study manuscript and led the health economic aspects. LM: contributed to the study design, protocol development, data analysis and redrafting the study manuscript and co-led the infectious diseases blinded outcome evaluation. EM: contributed to the study design, data collection and redrafting the study manuscript. KO: contributed to the study design, protocol development, including randomisation sequence and redrafting the study manuscript. SP was the overall project manager and contributed to the study design, protocol development, data collection, analysis and redrafting the study manuscript. ER co-led the infectious diseases blinded outcome assessment and contributed to the study design, protocol development, data analysis and redrafting the study manuscript. AR: contributed to the study design, protocol development, data analysis and redrafting the study manuscript with specific input into health economics. DW: contributed to the study statistical design, protocol development, led the statistical data analysis and redrafting the study manuscript. FW: contributed to the study design, protocol development, data analysis and redrafting the study manuscript.

Availability of data and material
Requests for data should be made to the corresponding author. Each request requires a research proposal including a clear research question and proposed analysis plan. Requests will be considered on an individual basis and are subject to review and approval by the ROCIT management committee and relevant human research ethics committees.

Compliance with ethical standards

Conflicts of interest
The authors report no conflicts of interest.

Ethical approval
The protocol was prospectively approved by the human research ethics committee of all participating institutions and reported prior to completion of the study. This was the South Metropolitan Health Service Human Research Ethics Committee ref:RGS000004, and the St John of God Health Care Human Research Ethics Committee ref:1183.

Consent to participate
Consent to participate was provided prospectively from all participants or their legal surrogate.

Consent for publication
The signed consent forms for all participants included consent to publication of aggregate data. The authors all consent to publication of the manuscript.

Code availability
Requests for code should be made to the corresponding author and will be considered on an individual basis by the study management committee.

Publisher's Note

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