Association between butyrate-producing gut bacteria and the risk of infectious disease hospitalisation: results from two observational, population-based microbiome studies

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Summary

Background Microbiota alterations are common in patients hospitalised for severe infections, and preclinical models have shown that anaerobic butyrate-producing gut bacteria protect against systemic infections. However, the relationship between microbiota disruptions and increased susceptibility to severe infections in humans remains unclear. We investigated the relationship between gut microbiota and the risk of future infection-related hospitalisation in two large population-based cohorts.

Methods In this observational microbiome study, gut microbiota were characterised using 16S rRNA gene sequencing in independent population-based cohorts from the Netherlands (HELIUS study; derivation cohort) and Finland (FINRISK 2002 study; validation cohort). HELIUS was conducted in Amsterdam, Netherlands, and included adults (aged 18–70 years at inclusion) who were randomly sampled from the municipality register of Amsterdam. FINRISK 2002 was conducted in six regions in Finland and is a population survey that included a random sample of adults (aged 25–74 years). In both cohorts, participants completed questionnaires, underwent a physical examination, and provided a faecal sample at inclusion (Jan 3, 2013, to Nov 27, 2015, for HELIUS participants and Jan 21 to April 19, 2002, for FINRISK participants. For inclusion in our study, a faecal sample needed to be provided and successfully sequenced, and national registry data needed to be available. Primary predictor variables were microbiota composition, diversity, and relative abundance of butyrate-producing bacteria. Our primary outcome was hospitalisation or mortality due to any infectious disease during 5–7-year follow-up after faecal sample collection, based on national registry data. We examined associations between microbiota and infection risk using microbial ecology and Cox proportional hazards.

Findings We profiled gut microbiota from 10 699 participants (4248 [39·7%] from the derivation cohort and 6451 [60·3%] from the validation cohort). 602 (5·6%) participants (152 [3·6%] from the derivation cohort; 450 [7·0%] from the validation cohort) were hospitalised or died due to infections during follow-up. Gut microbiota composition of these participants differed from those without hospitalisation for infections (derivation p=0·041; validation p=0·0002). Specifically, higher relative abundance of butyrate-producing bacteria was associated with a reduced risk of hospitalisation for infections (derivation cohort cause-specific hazard ratio 0·75 [95% CI 0·60–0·94] per 10% increase in butyrate producers, p=0·013; validation cohort 0·86 [0·77–0·96] per 10% increase, p=0·0077). These associations remained unchanged following adjustment for demographics, lifestyle, antibiotic exposure, and comorbidities.

Interpretation Gut microbiota composition, specifically colonisation with butyrate-producing bacteria, was associated with protection against hospitalisation for infectious diseases in the general population across two independent European cohorts. Further studies should investigate whether modulation of the microbiome can reduce the risk of severe infections.

Funding Amsterdam UMC, Porticus, National Institutes of Health, Netherlands Organisation for Health Research and Development (ZonMw), and Leducq Foundation.

Introduction

Infectious diseases continue to be a substantial global burden of disease despite major progress in prevention and treatment. The Global Burden of Disease study estimated that in 2019 approximately 25% of all deaths worldwide were due to infections.[1] This burden highlights the need for new preventive strategies. Patients hospitalised for infectious diseases often display gut perturbations, even before antibiotic treatment. We and others have shown that these patients have lower abundances of intestinal anaerobes and

Lancet Microbe 2024

Published Online https://doi.org/10.1016/S2666-5247(24)00079-X

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Research in context

Evidence before this study

We searched PubMed from database inception to July 10, 2023, without language restrictions, using the search terms ("gut" OR "intestinal") AND ("microbiome" OR "microbiota") AND ("infection" OR "infectious") AND "risk" AND ("patients" OR "participants" OR "cohort" OR "men" OR "women") in the title or abstract. This search identified 656 articles. Most human studies described gut microbiome disruptions during hospitalisation with infectious diseases or intensive care unit stay. However, these disruptions could be both a cause or consequence. Microbiota alterations could be a consequence of the infection itself, for example caused by systemic inflammation or dietary changes; yet, disrupted microbiota might also be causative by increasing infection susceptibility through effects on mucosal and systemic immune responses. In selected patient populations highly vulnerable to infections (eg, during severe illness or following stem-cell transplantation), a loss of microbial diversity, overgrowth of Gram-negative bacteria, and low abundances of anaerobic bacteria producing the short-chain fatty acid butyrate were associated with an increased risk of (hospital acquired) infections. No studies have investigated the relationship between gut microbiota and the future risk of severe infectious diseases in the human general population. Moreover, small sample sizes and the effect of geographical and technical variation (eg, sequencing techniques and analytical approaches) raise questions about the generalisability of previous findings.

Added value of this study

Our results indicate that baseline gut microbiota composition (before infection onset) was related to the risk of hospitalisation for infectious diseases in two large independent population-based cohorts, with a total of 10 699 participants and 602 events (ie, hospitalisation or mortality due to any infectious disease). In both cohorts, gut colonisation with anaerobic butyrate-producing microbiota were associated with protection against hospitalisations for infectious diseases. By both multivariable Cox proportional hazards models and carefully matching cases (participants hospitalised for an infection) to controls (those without infection-related hospitalisation), we found that this relation was robust and did not change after adjusting for age, sex, ethnicity, lifestyle, recent antibiotic exposure, and comorbidities. This study corroborates findings in preclinical studies and selected patients at high risk of severe infections and, to our knowledge, is the first to provide evidence on the effect of anaerobic gut microbiota on infection susceptibility in the human general population.

Implications of all the available evidence

Higher representation of anaerobic butyrate-producing gut bacteria was associated with a reduced risk of future infection-related hospitalisations in the general population across different geographical locations. Our findings suggest that gut microbiota was a potentially modifiable risk factor for severe infections and interventional studies are warranted.

increased amounts of potentially pathogenic gut bacteria.[2–4] It is currently unclear if these alterations are a consequence of the disease itself (for example caused by systemic inflammation or dietary changes), or that disrupted gut microbiota increase the susceptibility to infections from the outset. Mouse models have supported the latter hypothesis and shown that perturbation of commensal microbiota (by using antibiotic-treated or germ-free mice) blunted inflammatory responses and increased the susceptibility to and severity of infections.[5,6] The beneficial systemic effects of the microbiome are often attributed to metabolites produced by commensal anaerobic bacteria. For example, preclinical research showed that the microbiome-derived metabolite butyrate increases the antimicrobial activity of monocytes and affects the immunological environment in the lung.[2,7,8]

Our group previously described an association between depletion of anaerobic gut bacteria producing butyrate and an increased risk of respiratory infections in selected patients at a high risk of developing infections (eg, following a stroke or allogeneic haematopoietic stem-cell transplantation).[9,10] The beneficial effects of anaerobic, butyrate-producing bacteria might extend beyond such highly susceptible populations. Large epidemiologic studies described a 65–70% higher risk of re-admission with severe sepsis after hospitalisations with presumed microbiota disruptions, such as *Clostridioides difficile* infection or extensive antibiotic treatment, compared with hospitalised patients without such exposures.[11,12] However, these studies did not actually characterise the microbiome. Hence, evidence supporting the relation between gut microbiota and the risk of systemic infections is extrapolated from non-human research, and human data are limited to circumstantial evidence or selected populations at high risk. Whether variation in microbiota composition is associated with the susceptibility to infections in the general population remains unknown. Moreover, external validation in gut microbiota studies is nearly always absent, whereas geographical and technical variation might affect findings.

Here, we describe the association between gut microbiota characteristics and the risk of hospital admission for any infectious disease in the general population using data from two independent large-scale population-based cohort studies: the Dutch Healthy Life in an Urban Setting (HELIUS) study (derivation cohort), and the Finnish FINRISK 2002 population survey (validation cohort). We hypothesised that gut microbiota diversity and composition, specifically higher abundances of butyrate-producing bacteria, are associated with a reduced risk of hospital admission for infections among the general population.

Methods

Study design and participants

We conducted this study in two independent observational cohorts: the HELIUS study from the Netherlands, and FINRISK 2002 conducted in Finland. HELIUS served as the derivation cohort and FINRISK as validation. Details on our study cohorts are provided in the appendix (p 2) and have been previously described.[13–16] Briefly, HELIUS is a multi-ethnic, population-based prospective cohort study conducted in Amsterdam, Netherlands. Adults (aged 18–70 years at inclusion) were randomly sampled from the municipality register of Amsterdam, stratified by ethnicity, and invited to participate. HELIUS participants were linked to national hospitalisation and mortality registries, covering admissions to all Dutch hospitals and deaths from Jan 1, 2013, to Dec 31, 2020. The FINRISK 2002 cohort is a population survey that included a random sample of adults (aged 25–74 years) from six regions in Finland, stratified by sex, region, and 10-year age group. FINRISK participants were linked to the Finnish Hospital Discharge and Causes-of-Death Registers. In both cohorts, participants completed questionnaires, underwent a physical examination, and provided faecal samples at inclusion (Jan 3, 2013, to Nov 27, 2015, for HELIUS participants and Jan 21 to April, 19, 2002, for FINRISK participants). Dietary variables were available in a subset of 705 HELIUS participants. For inclusion into our study, a faecal sample needed to be provided and successfully sequenced, and registry needed to be available (appendix p 2). The date range for recruitment to the present study was the same as the HELIUS and FINRISK recruitment dates.

This study conformed to the STORMS guidelines (appendix pp 27–29).[17] Ethical approval for HELIUS was obtained from the Academic Medical Center Ethical Review Board (protocol number: 10/100; amendment 10/100#10.17.1729; NL32251.018.10) and ethical approval for FINRISK from the Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital district (reference 558/E3/2001). No separate ethical approval was needed for the present study. All participants in the HELIUS and FINRISK studies provided written informed consent.

Procedures

Details on faecal sample collection, DNA extraction, microbiota sequencing, and preprocessing have been previously described and are provided in the appendix (pp 2–3).[14,16,18,19] In summary, a repeated bead-beating method was used for DNA extraction in HELIUS participants. DNA extraction for FINRISK participants followed Earth Microbiome Project protocols. In both cohorts, the V4 region of the 16S ribosomal RNA gene was sequenced on an Illumina system (San Diego, CA, USA) and further preprocessing of sequence data was performed by uniform methods using Deblur (version 1.1.1) and Greengenes2 (release 2022.10).

Data on hospitalisations and mortality were retrieved from national registries (appendix pp 3–4). We used ICD-10 codes to identify the primary reason (diagnosis) for hospitalisation or mortality. ICD-10 codes were classified as infectious disease before data retrieval based on a study published in 2021.[20] Details on national registry data and ICD-10 classification are provided in the appendix (pp 3–4).

See Online for appendix

Outcomes

The primary outcome was hospital admission or mortality due to any infectious disease during follow-up after faecal sample collection at study inclusion to HELIUS (5–7 years' follow-up) or FINRISK (6 years' follow-up). Mortality due to non-infectious diseases was treated as a competing risk. For participants with multiple admissions for infections, only the first admission was considered.

Statistical analysis

Our statistical methods are detailed in the appendix (pp 5–7). We assessed associations between our outcome variable and predefined key features of the bacterial gut microbiome: community composition, α-diversity (quantified using the Shannon Diversity Index), and the relative abundance of butyrate-producing bacteria (given butyrate's well established effects in animal experiments). The amount of butyrate-producing bacteria was calculated based on the cumulative relative abundance of 16 bacteria known to be the most abundant drivers of butyrate production (appendix p 12), as previously described and of which the accuracy to predict actual faecal butyrate concentrations has been independently confirmed.[9,10,21] Sensitivity analyses included (1) expanding our outcome to all admissions with infectious diseases, rather than admissions with an infection as primary diagnosis, and (2) using an alternative list of butyrate producers to calculate amounts of butyrate-producing bacteria.[22] Differences in community composition were assessed using permutational multivariate ANOVA (β-diversity using Bray–Curtis dissimilarities). To identify bacteria driving differences in composition between groups, DESeq2 (version 1.30.1) and Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) were used. An individual microbiota-based risk score for infection-related hospitalisations was derived from the HELIUS cohort by calculating a weight for each bacterium using regularised Cox regression with 10-fold cross-validation.[23] For validation, risk scores for FINRISK participants were calculated by multiplying HELIUS-derived weights with participant-specific relative abundances. Competing risk regression models were used to assess associations between outcomes and continuous microbiota features (diversity, butyrate-producing bacteria, and risk score). We calculated cause-specific hazard ratios (csHRs), treating infection (both hospitalisation and mortality) as an outcome and non-infectious disease mortality as a competing risk. When butyrate producers were analysed as a continuous variable, csHRs refer to the risk per 10% increase in relative abundances of butyrate producers. Day 0 was defined as the day of faecal sample collection. Multivariable models were adjusted for baseline age, sex, ethnicity

	HELIUS cohort (derivation; n=4248)	FINRISK cohort (validation; n=6451)
Age, years	51·0 (41·0–58·0)	50·0 (38·8–59·0)
Sex		
Male	2044 (48·1%)	2945 (45·7%)
Female	2204 (51·9%)	3506 (54·3%)
Ethnicity*		
Dutch	1144 (26·9%)	..
African Surinamese	920 (21·7%)	..
South-Asian Surinamese	627 (14·8%)	..
Turkish	504 (11·9%)	..
Moroccan	712 (16·8%)	..
Ghanaian and other	341 (8·0%)	..
BMI kg/m^2	26·7 (23·9–30·2)	26·3 (23·7–29·4)
Current or former smoker	1986 (46·8%)	2998 (46·5%)
Alcohol usage†	2340 (55·1%)	5732 (88·9%)
Recent antibiotic exposure‡	394 (9·3%)	825 (12·8%)
Comorbidities		
Hypertension	940 (22·1%)	3076 (47·7%)
Diabetes	446 (10·5%)	348 (5·4%)
Cardiovascular disease	310 (7·3%)	230 (3·6%)
Pulmonary disease	398 (9·4%)	537 (8·3%)
Gastrointestinal disease	216 (5·1%)	102 (1·6%)
Cancer	85 (2·0%)	232 (3·6%)
Follow-up time, years§	5·55 (5·30–6·72)	6·00 (6·00–6·00)
Infectious disease-related hospitalisation or mortality	152 (3·6%)	450 (7·0%)¶
Lower respiratory tract infection	48 (31·6%)	100 (22·2%)
Abdominal infection	37 (24·3%)	88 (19·6%)
Urinary tract infection	15 (9·9%)	35 (7·8%)
Skin infection	14 (9·2%)	9 (2·0%)
Infection following surgery or medical procedure	13 (8·6%)	31 (6·9%)
Other	25 (16·4%)	189 (42·0%)

Data are n (%) or median (IQR). *Ethnicity was not recorded in the FINRISK cohort. †In both the HELIUS and FINRISK cohorts, alcohol usage was self-reported (any or none). ‡Antibiotic exposure during the 3 months before faecal sample collection for HELIUS participants and 4 months before for FINRISK participants. §Time from sample collection to end of study, irrespective of whether an event occurs. Based on the follow-up duration in the HELIUS study, follow-up for the FINRISK cohort was limited to 6 years following sample collection. ¶Two participants from the FINRISK cohort simultaneously had two types (ie, anatomic sources) of infection.

Table 1: **Characteristics of the HELIUS and FINRISK cohorts**

(HELIUS only), smoking, alcohol use, physical activity, antibiotic exposure (3 months before sample collection for HELIUS participants and 4 months for FINRISK participants), and comorbidities (hypertension, diabetes, cancer, cardiovascular disease, pulmonary disease, and gastrointestinal disease). Nested matched case–control analyses within the derivation cohort were performed as supplemental analysis to examine differences in gut microbiota between pairs of HELIUS participants with an infection-related hospitalisation (cases) and those without infection-related hospitalisation (controls), matched on age, sex, ethnicity, antibiotic exposure, diabetes, and pulmonary and gastrointestinal comorbidities. Missing data were minimal and included as a categorical variable in multivariable analyses. Continuous data are presented as median with interquartile range. Categorical variables were summarised as percentages and compared with the χ^2 test. Two-tailed level of significance was set at (adjusted) p of less than 0·05. Statistical analyses were performed in the R statistical framework (version 4).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Gut microbiota of 10 699 participants, 4248 (39·7%) from the HELIUS study (derivation cohort, Netherlands) and 6451 (60·3%) from FINRISK (validation cohort, Finland) were characterised and used for the present study. Demographic and clinical characteristics of the 10 699 included participants are in table 1. Participants were linked to national registries to identify hospital admissions for infectious diseases during follow-up. A flowchart of study inclusions is in the appendix (p 14). In both cohorts, gut microbiota predominantly consisted of Firmicutes (also known as Bacillota; mean relative abundance of 65·9% [SD 12·6] in the derivation cohort and 62·2% [15·4] in the validation cohort) and Bacteroidetes (mean relative abundance 24·1% [13·2] in the derivation cohort and 27·6% [15·5] in the validation cohort; appendix p 15). Between sample collection and Dec 31, 2020, 152 (3·6%) participants of the HELIUS study were hospitalised or died due to an infectious disease. In the FINRISK study, 450 (7·0%) participants were hospitalised or died due to an infection during 6-year follow-up. Lower respiratory tract infections were the most common reason for infection-related hospitalisations in both cohorts (table 1).

We first examined whether the community composition of gut microbiota differed between HELIUS participants who reached the primary outcome (hospitalisation or mortality due to an infection) and those without hospitalisation due to infectious diseases (including participants who died from non-infectious causes). There was a detectable separation between these outcome groups (figure 1A), which was confirmed statistically via permutation testing (R^2=0·00035, p=0·041). This separation was robust at higher taxonomic levels (genus level p=0·040; family level p=0·024) and with phylogenetically-aware distances (weighted UniFrac p=0·040), although the effect size was small—even when considering that multiple classic determinants together (demographics, diet, comorbidities, medication, socioeconomic, and technical factors) commonly explain little variance in microbiota composition (R^2=0·09–0·12).[14,24] The relative abundance of butyrate-producing bacteria was associated with microbiota composition, as visualised by colour-coding the principal coordinates analysis (p=0·0010; figure 1B). Using a DESeq2 model to identify specific bacteria responsible for differences between outcome groups, we found higher relative abundances of *Veillonella* and *Streptococcus* in participants hospitalised for infections. Participants without hospitalisation due to infectious diseases had higher levels of obligate anaerobic bacteria

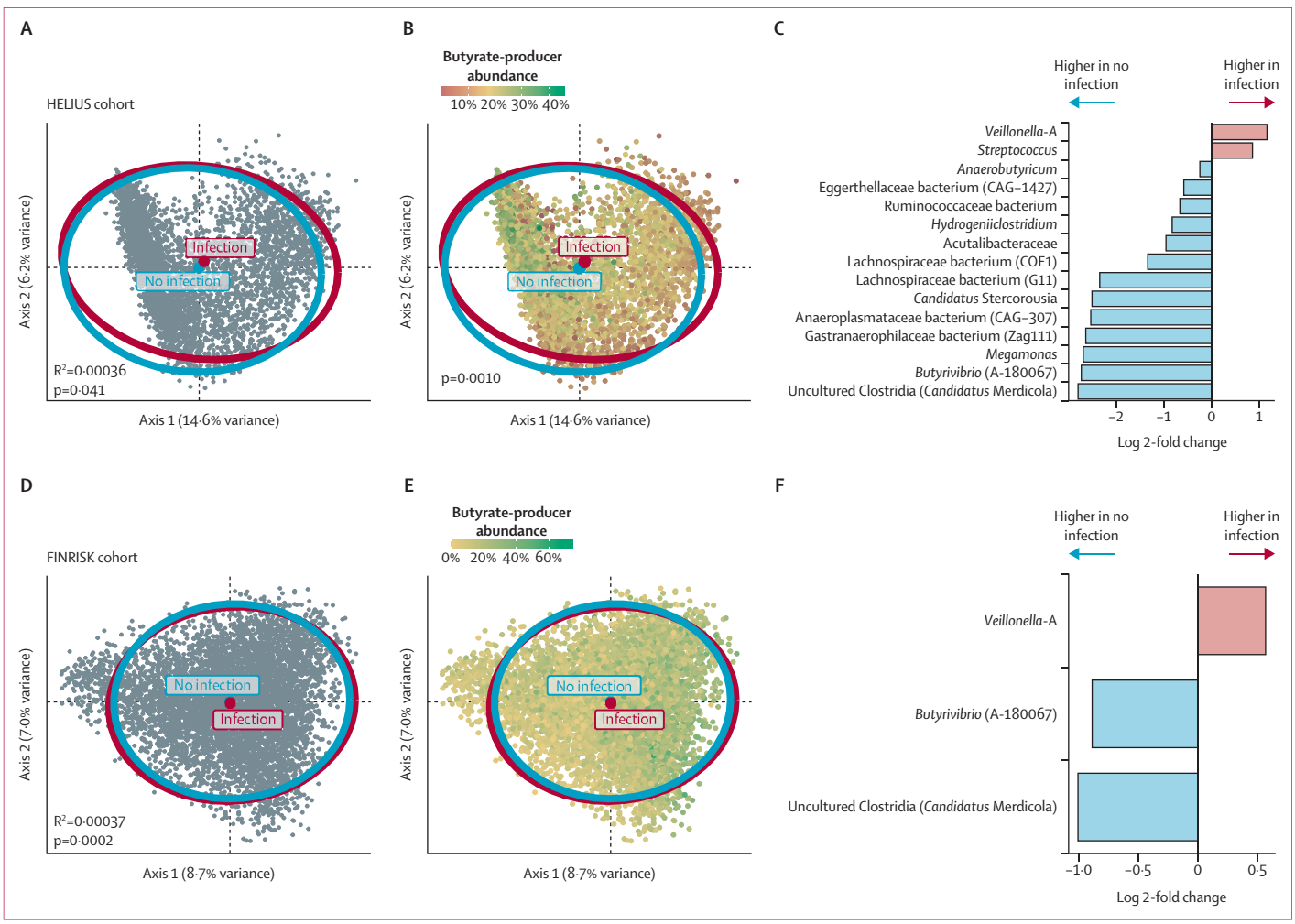

Figure 1: **The association of gut microbiota community composition and the risk of hospitalisation for infectious diseases**

(A) In the HELIUS cohort (derivation), gut microbiota composition (β-diversity) differed between participants hospitalised with an infection during follow-up (n=152) and those without hospitalisation for infectious diseases (n=4096). Significance of differences in community composition between these groups is determined using permutational multivariate ANOVA with Bray–Curtis dissimilarities at the level of amplicon sequence variants. Each grey point represents a HELIUS participant, red and blue points represent the group centres, and ellipses represent the 95% CI. Visualised using principal coordinate analysis. (B) Colour-coding points according to the relative abundance of butyrate-producing bacteria showed that these bacteria are linked with the clustering of samples. Permutational multivariate ANOVA with Bray–Curtis dissimilarities at the level of amplicon sequence variants was used to determine significance of the association between the relative abundance of butyrate-producing bacteria and community composition. (C) The compositional difference between outcome groups was driven by higher relative abundances of *Veillonella* and *Streptococcus* spp in participants with infection-related hospital admissions, and lower relative abundances of anaerobic bacteria (eg, *Butyrivibrio*), as identified by a DESeq2 model. (D) Similar to the derivation cohort, gut microbiota composition of FINRISK participants (validation cohort) with an infection during follow-up (n=450) differed from those without (n=6001). (E) Contribution of butyrate producers to microbiota composition. (F) Upon validation from differentially abundant genera in the derivation cohort (depicted in panel C), higher relative abundances of *Veillonella* and lower relative abundances of *Butyrivibrio* and uncultured Clostridia (*Candidatus* Merdicola) were also identified in FINRISK participants with infection-related admissions.

(eg, *Butyrivibrio*; figure 1C). Similar results were obtained when using ANCOM-BC instead of DESeq2 (appendix p 16). In the validation cohort (FINRISK), we also observed a difference in gut microbiota between participants hospitalised for an infection and those not hospitalised for an infection (R^2=0·00037, p=0·0002; figure 1D, E). In concordance with the derivation cohort, participants from the validation cohort hospitalised for infections had lower relative abundances of *Butyrivibrio* and higher relative abundances of *Veillonella* (figure 1F). Together, these data showed that, in two independent cohorts, baseline gut microbiota composition differed between participants hospitalised with an infection during follow-up and those without infection-related hospitalisation, similarly driven by an increase in *Veillonella* and decrease of the obligate anaerobe *Butyrivibrio*.

Permutation testing and DESeq2 only provide information on group-level microbiota differences, rather than the association between microbiota composition and an individual's risk. We therefore sought to identify a signature of bacterial abundances associated with the risk of infectious disease hospitalisation. Regularised Cox regression was performed in the derivation cohort to derive a risk score.[23] At independent validation, FINRISK participants with high

	Number of participants	Number of infections	Univariable analysis		Multivariable analysis	
			csHR (95% CI)	p value	csHR (95% CI)	p value
Derivation cohort diversity						
Continuous	4248	152	0·72 (0·52–0·98)	0·038	0·88 (0·61–1·28)	0·51
Low tertile	1416	62	1 (ref)	..	1 (ref)	..
High tertile	1416	43	0·72 (0·49–1·06)	0·099	0·91 (0·57–1·43)	0·67
Validation cohort diversity						
Continuous	6451	450	0·73 (0·60–0·90)	0·0024	0·81 (0·66–0·997)	0·047
Low tertile	2151	172	1 (ref)	..	1 (ref)	..
High tertile	2150	139	0·80 (0·64–0·996)	0·046	0·89 (0·71–1·11)	0·30

Multivariable cause-specific Cox proportional hazards analyses of the association between gut microbiota diversity (quantified by the Shannon Diversity Index) and hospitalisation for an infectious disease. Multivariable models are adjusted for age, sex, ethnicity (derivation cohort only), smoking, alcohol usage, physical activity, antibiotic exposure in the 3 months (derivation cohort) or 4 months (validation cohort) before faecal sample collection, and comorbidities (hypertension, diabetes, cardiovascular disease, cancer, pulmonary disease, and gastrointestinal diseases). Distinct multivariable analyses were done for gut microbiota diversity as a continuous variable and as categorical variables (in tertiles). csHR=cause-specific hazard ratio.

Table 2: **Association of gut microbiota diversity with the risk of hospitalisation for infection**

values of this score (highest tertile) had an increased risk of hospitalisation due to infectious diseases compared with participants in the lowest tertile (csHR 1·39 [95% CI 1·10–1·75], p=0·0060; c-statistic 0·53 [SE 0·013]; appendix p 17), indicating that a signature of specific bacterial abundances was informative about the risk of infection-related hospitalisation in the general population across cohorts.

Gut microbiota diversity was associated with the primary outcome in the derivation (csHR 0·72 [95% CI 0·52–0·98] per point increase in Shannon diversity, p=0·038), and validation cohort (0·73 [0·60–0·90], p=0·0024). This association was not significant in multivariable analysis in the derivation cohort (0·88 [0·61–1·28], p=0·51), albeit significant in the validation cohort (0·81 [0·66–0·997], p=0·047; table 2).

We next asked if the relative abundance of butyrate-producing bacteria was associated with the risk of severe infection (hospitalisation or mortality). Among the 4248 participants of the derivation cohort, increased relative abundances of butyrate-producing bacteria were associated with a lower risk of future hospitalisation for infectious diseases (csHR 0·75 [95% CI 0·60–0·94], p=0·013; figure 2A, B; table 3). In other words, for each additional 10% increase in the relative abundance of butyrate-producing bacteria, the csHR of infection-related hospitalisation was 0·75. This association was also observed when tertiles of butyrate producers were compared: 42 severe infections in 1416 participants in the highest tertile versus 69 severe infections in 1416 participants in the lowest tertile (0·62 [0·42–0·91], p=0·014; figure 2A, B; table 3). In the validation cohort, the same association between higher relative abundances of butyrate-producing bacteria and a lower risk of hospitalisation for infections was observed (0·86 [0·77–0·96], p=0·0077, per 10% increase; figure 2C, D). In both the derivation and validation cohort, correction for potential confounders (age, sex, ethnicity, smoking, alcohol usage, physical activity, antibiotic exposure, and comorbidities) in multivariable analyses did not substantially impact effect estimates nor significance (table 3). Given the proposed bi-directional relationship between gut microbiota and obesity, BMI was not considered a true confounder. Yet, when BMI was additionally included in the multivariable model, the association was unaltered (derivation 0·78 [0·62–0·98], p=0·033; validation 0·88 [0·79–0·97], p=0·015). Neither did correcting for the compositional nature of microbiome-data using a centred log-ratio transformation affect these findings (appendix p 18). We thus concluded that colonisation with butyrate-producing bacteria was associated with a decreased risk of severe infections in both the derivation and validation cohort.

In sensitivity analysis within the derivation cohort, any hospitalisation with an infection was considered an event (rather than an infection as primary diagnosis). A similar association between gut microbiota and the risk of hospitalisation with an infection was observed (appendix pp 19–20). Additionally, recalculating relative abundances of butyrate-producing bacteria using an alternative approach[24] also yielded results similar to the main analyses (appendix p 21), showing the robustness of the microbiota-infection association. In a nested, matched, case–control analysis in the derivation cohort, HELIUS participants hospitalised for infection during follow-up (cases) were matched in a 1:1 ratio to HELIUS participants without hospitalisation due to infectious diseases (controls) on age, sex, ethnicity, antibiotic exposure, diabetes, and pulmonary and gastrointestinal comorbidities (appendix p 22 for clinical characteristics). Gut microbiota diversity of cases and controls did not differ (p=0·34; appendix p 23). Yet, consistent with the data from the full cohort, participants with a hospitalisation due to infectious diseases during follow-up had lower baseline relative abundances of butyrate-producing bacteria (median difference between matched cases and controls: –3·0% [IQR –12·3 to 5·8], p=0·0007), and overall distinct gut microbiota composition compared with matched controls (p=0·038; appendix p 23).

In further exploratory analysis, we examined whether demographics, lifestyle factors, antibiotics, and comorbidities changed the effect estimates of butyrate producers on infection risk by computing contrasts. Although several covariates (such as sex, age, BMI, and diabetes) were associated with the relative abundance of butyrate-producing bacteria and the risk of hospitalisation due to infectious diseases, the relationship between butyrate producers and hospitalisation due to infectious diseases was not moderated by most covariates. Yet, in participants with a BMI of 30 km/m^2 or more, the association between butyrate producers and risk of infection-related hospitalisation seemed absent. Of note, no interaction between the microbiota-infection association and ethnicity was observed, nor were dietary variables strongly associated with butyrate-producing bacteria (appendix pp 24–26).

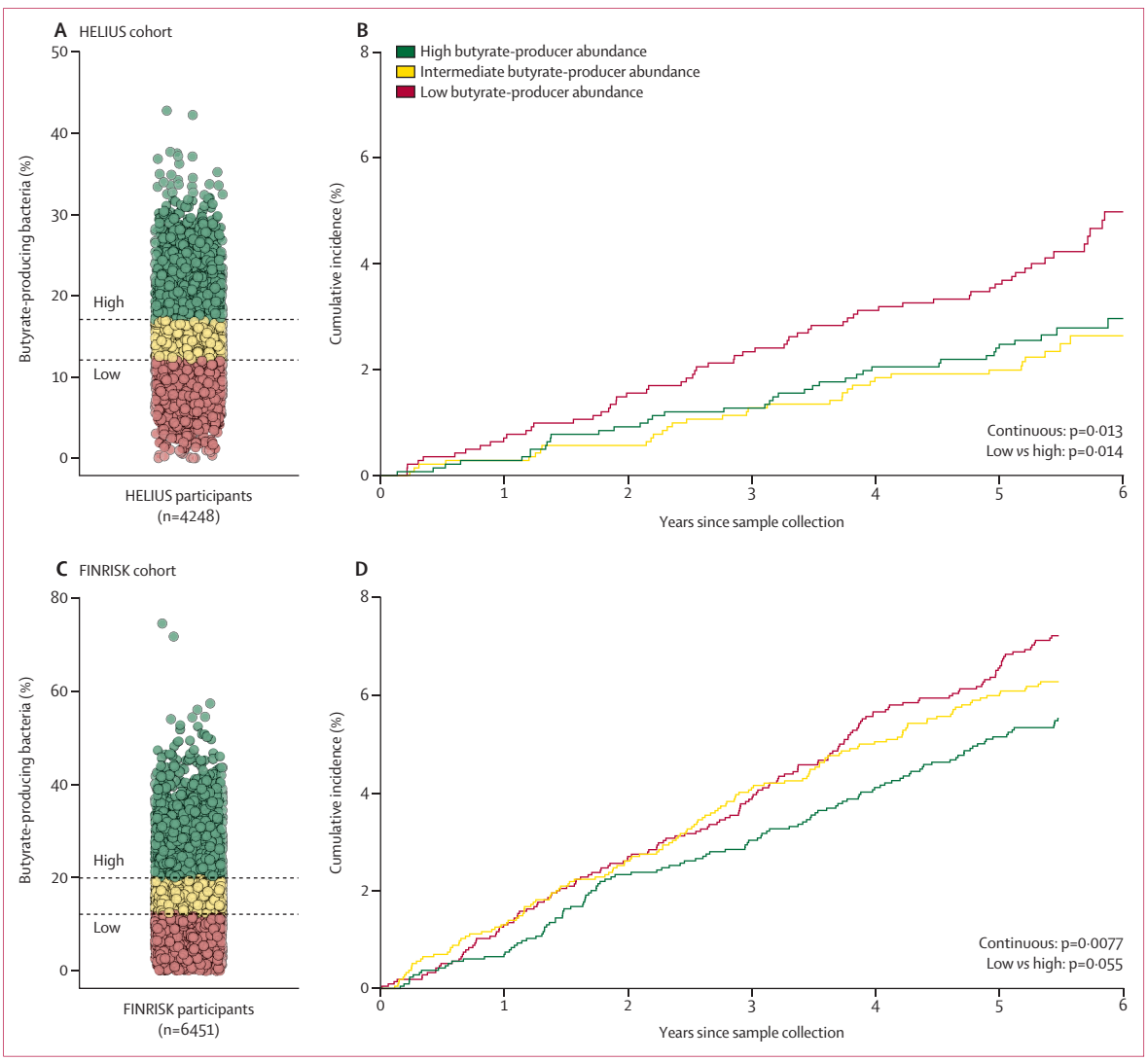

Figure 2: **Colonisation with butyrate-producing bacteria is associated with protection against hospitalisation for infectious diseases**
(A) Dot plot showing the relative intestinal abundance of butyrate producers in the HELIUS cohort (derivation; n=4248). (B) Cumulative incidence of severe infection (either hospital admission or mortality) in the HELIUS cohort, stratified by tertiles of butyrate-producer abundance. Participants with lower relative abundances of butyrate-producing bacteria had an increased risk of hospitalisation or mortality due to an infection compared with those with high abundances. (C) Dot plot showing the relative intestinal abundance of butyrate producers in the FINRISK cohort (validation; n=6451). (D) Similar to the derivation cohort, participants in the validation cohort with lower relative abundances of butyrate-producing bacteria had an increased risk of hospitalisation or mortality due to an infection compared with those with higher abundances. Hypothesis testing was done using competing risk regression models (with non-infectious disease mortality as competing risk). Butyrate producers were considered separately as either a continuous variable or as cohort-specific tertile-stratified categories.

Discussion

In these independent large observational cohorts (HELIUS and FINRISK), we showed that gut microbiota was associated with the risk of infectious disease-related hospitalisation and mortality in the general population. Higher abundance of anaerobic butyrate-producing bacteria was associated with protection against severe infections, even when adjusted for demographics, lifestyle, antibiotic exposure, and comorbidities. Despite the differences between cohorts in participants' characteristics, microbiota characterisation methods, and infection rates, we observed similar associations between gut microbiota and the risk of infection-related hospitalisation across geographical locations (ie, the Netherlands and Finland). To the best of our knowledge, this is the largest study linking gut microbiota to severe infection susceptibility in humans, and the first to identify an association between butyrate-producing bacteria and favourable long-term outcomes, outside of animal experiments or groups at high risk. Our findings suggest that gut microbiota might be a potentially modifiable risk factor for infection-related hospitalisations and interventional studies are warranted.

Gut microbiota are often disrupted in patients hospitalised for severe infections, which has been associated with clinical

	Number of participants	Number of infections	Univariable analysis		Multivariable analysis	
			csHR (95% CI)	p value	csHR (95% CI)	p value
Derivation cohort butyrate producers						
Continuous	4248	152	0·75 (0·60–0·94)	0·013	0·77 (0·62–0·97)	0·025
Low tertile	1416	69	1 (ref)	..	1 (ref)	..
High tertile	1416	42	0·62 (0·42–0·91)	0·014	0·60 (0·40–0·89)	0·012
Validation cohort butyrate producers						
Continuous	6451	450	0·86 (0·77–0·96)	0·0077	0·90 (0·81–0·99)	0·040
Low tertile	2151	168	1 (ref)	..	1 (ref)	..
High tertile	2150	136	0·80 (0·64–1·01)	0·055	0·86 (0·68–1·08)	0·19

Multivariable cause-specific competing risk regression analyses of the association between the relative abundance of butyrate-producing gut bacteria and hospitalisation for an infectious disease. Multivariable models are adjusted for age, sex, ethnicity (derivation cohort only), smoking, alcohol usage, physical activity, antibiotic exposure in the 3 months (derivation cohort) or 4 months (validation cohort) before faecal sample collection, and comorbidities (hypertension, diabetes, cardiovascular disease, cancer, pulmonary disease, and gastrointestinal diseases). Distinct multivariable analyses were done for butyrate producers as a continuous variable (per 10% increase) and as categorical variables (stratified into tertiles). csHR=cause-specific hazard ratio.

***Table 3*: Association of intestinal butyrate-producing bacteria with infection**

outcomes.[2–4] However, these disruptions could be either a consequence of disease, or precede the infection and affect susceptibility. Here, we showed that gut microbiota, characterised before infection onset, differed between participants hospitalised for infections during follow-up and those without hospitalisation for infections, indicating that gut microbiota was associated with the susceptibility to severe infections. In independent cohorts, this difference in microbiota composition between outcome groups was identically driven by an increase in *Veillonella* (a genus that includes opportunistic pathogens associated with an increased risk of developing severe symptoms during SARS-CoV-2 infections and enriched in hospitalised patients with COVID-19),[3,25] a decrease of *Butyrivibrio* (a butyrate-producing genus), and a strict anaerobic uncultured Clostridia (*Candidatus* Merdicola), of which there is little current knowledge.

Preclinical studies showed that the microbial metabolite butyrate enhances protection against infections while preventing immune-associated pathology. Butyrate induces the production of antimicrobial peptides, increases colonisation resistance, and reduces neutrophil recruitment to the airways during influenza infection in mice, thereby limiting tissue damage.[2,7,8] Given this well established effect of butyrate on mucosal and systemic immunity in animal experiments, we investigated the relationship between relative abundances of butyrate-producing bacteria (identified through a previously validated approach)[9,10,21] and infection susceptibility in humans. We found that higher representation of intestinal butyrate producers was associated with protection against infection-related hospitalisations, independent from potential confounders in both multivariable and strictly matched analyses, and across geographical locations. Our findings corroborate the previously described effect of anaerobic microbiota depletion on infection risk and adverse clinical outcomes in selected groups at high risk.[9,10,26] For example, we and others showed that reduction of butyrate-producing bacteria (which are exclusively strict anaerobes) increased the risk of infection following stroke, kidney transplantation, and allogeneic haematopoietic stem-cell transplantation,[9,10,26] and anaerobic microbiota (before infection) were associated with less symptoms during COVID-19.[25] Moreover, a study published in 2023 that combined observational data of 3032 mechanically ventilated patients with animal modelling showed that early treatment with antianaerobic antibiotics decreased overall and infection-free survival.[27] We confirmed these adverse effects of antianaerobic antibiotics in 15 908 emergency department patients.[28] Interestingly, depletion of anaerobic microbiota by such antibiotics resulted in expansion of *Enterobacteriaceae* and our consortium previously identified associations between *Enterobacteriaceae*-related microbiota signatures and all-cause mortality in the general population.[16,27] Taken together, these findings show the importance of anaerobic, butyrate-producing gut microbiota in both patients at high risk of severe infections and the general population, and suggest that the widespread use of antianaerobic antibiotics should be reconsidered.[27,28]

Strengths of this study include the large, representative, multi-ethnic population cohorts, comprehensive follow-up information, and external validation. Independent validation is absent in most microbiome studies, and ethnicity (which reflects differences in, among others, diet, lifestyle, environment, and genetics) and technical factors are main contributors to microbiota composition,[14,24] raising the question of whether associations are generalisable. Here, effects were similar associations across ethnic groups in the ethnically diverse HELIUS population and we were able to validate key findings in an independent cohort, regardless of differences in microbiota sequencing methods, underlying diseases, and infection-related hospitalisation rates. Despite such heterogeneity, we found that a microbiota-based risk score derived in one cohort (HELIUS) was associated with the risk of severe infection in an independent cohort (FINRISK), suggesting that similar gut bacteria contribute to infection risk in the human general population across different geographical locations.

Our study has limitations. First, microbiota were characterised at a single timepoint. Gut microbiota might change over time, potentially obscuring the effect on outcomes at distant timepoints. Yet, individual microbiota composition is remarkably stable, with the majority of strains remaining stable over 5 years and many bacteria are residents for decades.[29] Second, the potentially beneficial effects of butyrate-producing bacteria might extend beyond butyrate as several butyrate producers are capable of the biosynthesis of secondary bile acids and other metabolites with potential immunomodulatory effects.[30] Similarly, butyrate producers might have distinct effects in bacterial, viral, and fungal infections. Additional experimental and functional studies will be necessary to establish which mechanisms contribute to the protective effects of anaerobic gut bacteria. Furthermore, as with all observational studies, our findings do not necessarily prove causality. For example, we could not

exclude diet as a confounder, although gut microbiota might also mediate the effects of diet on infection risk. Similarly, the antimicrobial spectrum (eg, having antianaerobic activity or not) was not considered when controlling for antibiotic exposure. However, lower butyrate-producing bacteria abundances were associated with the risk of severe infections in both matched case–control analysis and multivariable models that accounted for many confounding factors (including demographics, lifestyle, and comorbidities). Moreover, our findings are consistent with preclinical studies showing the protective effect of anaerobic microbiota against infections.[2,7,8,27]

In conclusion, we showed that gut microbiome composition, specifically colonisation with anaerobic butyrate-producing bacteria, was associated with a reduced risk of hospitalisation for infectious diseases. This study defines potential opportunities for interventional studies evaluating gut microbiota-directed therapies (such as targeted delivery of butyrate-producing bacteria or limiting gut anaerobe depletion) aiming to decrease the susceptibility to systemic infections.

Contributors

RFJK, BWH, HP-S, JMB, MIA-A, MP, AHMvdZ, B-JvdB, WMdV, and WJW conceived and designed the study. HG, ASH, JP, VS, DM, MP, B-JvdB, PJ, MN, and RK were involved in data collection and microbiota characterisation. RFJK, IW, BWH, ASH, AK, CB, LL, TN, and WJW analysed and interpreted the data. RFJK, IW, BWH, TN, and WJW wrote the manuscript. RFJK and BWH had access to and verified the underlying data of the HELIUS study. IW, ASH, AK, LL, and TN had access to and verified the underlying data of the FINRISK study. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

DM owns stock in and is a consultant for Biomesense. AHMvdZ reports grants from Health Hjolland, GSK, Boehringer Ingelheim, and Vertx; consulting fees from Boehringer Ingelheim and AstraZeneca; and honoraria from GSK, all outside the submitted work; and is a chair of the data safety monitoring board of a study on bronchopulmonary dysplasia in neonates. B-JvdB is the chair of the cardiovasular theme of HELIUS, the Netherlands Society of Hypertension, and Amsterdam Cardiovascular Sciences; and participates on the committee of the Dutch Cardiovascular Alliance. RK is on the Scientific Advisory Board of Gencirq, Cybele, DayTwo, Biomesense, and Micronoma; owns stock in Gencirq, Cybele, Biomesense, Micronoma, and Biota; is a consultant for DayTwo and Biomesense; and is a cofounder of Micronoma and Biota. MN is on the Scientific Advisory Board of Caelus Pharmaceuticals. TN reports grants from the Finnish Research Council, Emil Aaltonen Foundation, Sigrid Jusélius Foundation, and Finnish Foundation for Cardiovascular Research; and honoraria from Servier Finland and AstraZeneca. WJW reports grants from ZonMw/NWO and EU (Eurostars); and ad hoc consultancy for AstraZeneca and Shionogi, outside the submitted work. All other authors declare no competing interests.

Data sharing

Microbiota 16S rRNA gene sequence data from the HELIUS and FINRISK cohorts have been deposited in the European genome-phenome archive (HELIUS accession number EGAD00001004106; FINRISK accession number EGAD50000000287). Original code used for analysis is publicly available at https://github.com/rfjkullberg/microbiota_infections (https://doi.org/10.5281/zenodo.8146217). The HELIUS data are owned by the Academic Medical Center in Amsterdam, Netherlands. Data can be requested by submitting a proposal at http://www.heliusstudy.nl/en/ researchers/collaboration, which will be checked for compatibility with the general objectives, ethical approval, and informed consent forms of the HELIUS study. Data protection regulations in the Netherlands and Finland do not allow public sharing of individual participant data on hospitalisations or mortality. Dutch hospitalisation data (HELIUS) are accessible for statistical and scientific research under strict conditions through Statistics Netherlands (https://www.cbs.nl/en-gb/onze-diensten/customised-services-microdata/microdata-conducting-your-own-research). Finnish data (FINRISK) are available through the THL biobank upon submission of a research plan and signing a data transfer agreement (https://thl.fi/en/web/thl-biobank/for-researchers/application-process).

Acknowledgments

This study was supported by an Amsterdam University Medical Centers PhD scholarship to RFJK and a Niels Stensen Fellowship to BWH. RK is supported by a National Institutes of Health Pioneer grant (DP1-AT010885). MN is supported by a personal ZONMW-VICI grant 2020 (09150182010020) and a Leducq consortium grant (17CVD01). The HELIUS study is conducted by the Amsterdam UMC, location University of Amsterdam and the Public Health Service of Amsterdam. Both organisations provided core support for HELIUS. The HELIUS study was funded by the Dutch Heart Foundation under grant 2010T084; the Netherlands Organization for Health Research and Development under grant 200500003; the European Union FP-7 under grant 278901; and the European Fund for the Integration of non-EU immigrants under grant 2013EIF013. FINRISK is supported by the Finnish Foundation for Cardiovascular Research, the Emil Aaltonen Foundation, the Paavo Nurmi Foundation, the Sigrid Jusélius Foundation, the Juho Vainio Foundation, and the Academy of Finland (grants 321351 and 321356). We are most grateful to the participants of the HELIUS and FINRISK 2002 studies, and all staff involved in data collection.

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