Open Forum Infectious Diseases

MAJOR ARTICLE

Gut Microbiome in Human Melioidosis: Composition and Resistome Dynamics from Diagnosis to Discovery

Soumi Chowdhury,[1,2] Robert F. J. Kullberg,[3] Bastiaan W. Haak,[3,4] Claudio Duran,[4] Venkat A. Earny,[1,2] Vandana K. Eshwara,[1,2,5] Trevor D. Lawley,[4] W. Joost Wiersinga,[3,6] and Chiranjay Mukhopadhyay[1,2,5]

[1]Department of Microbiology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal 576104, Karnataka, India, [2]Center of Emerging and Tropical Diseases (CETD), Manipal Academy of Higher Education, Manipal 576104, Karnataka, India, [3]Center for Experimental and Molecular Medicine, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, The Netherlands, [4]Host-Microbiota Interactions Laboratory, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom, [5]Center for Antimicrobial Resistance and Education (CARE), Manipal Academy of Higher Education, Manipal 576104, Karnataka, India, and [6]Division of Infectious Diseases, Department of Internal Medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands

Background. Melioidosis, attributable to the soil-dwelling bacterium *Burkholderia pseudomallei*, stands as a paramount global health challenge, necessitating extended courses of antibiotics. While murine studies identified the gut microbiota as a modulator of bacterial dissemination during melioidosis, the human intestinal microbiota during melioidosis remains uncharacterized. Here, we characterized gut microbiota composition and antimicrobial resistance (AMR) genes at diagnosis, during treatment, and postdischarge for melioidosis. We hypothesized that the gut microbiota of melioidosis patients would be extensively distorted.

Methods. In this prospective observational cohort, stool samples of patients with culture-confirmed melioidosis admitted to a tertiary care hospital in India were collected at diagnosis, 14 days after diagnosis, or discharge (whichever occurred first) and at 6 months postinfection. Family members or neighbors served as community controls. The gut microbiota and resistome were profiled by shotgun metagenomic sequencing.

Results. We longitudinally analyzed the gut microbiota of 70 fecal samples from 28 patients and 16 community controls. At diagnosis, the gut microbiota of patients differed from that of controls, characterized by high abundances of potentially pathogenic bacteria, a loss of butyrate-producing bacteria, and higher levels of AMR genes. Microbiota composition and resistome remained different from community controls at 6 months, driven by total antibiotic exposure. During hospitalization, gut microbiota profiles were associated with secondary *Klebsiella pneumoniae* infections.

Conclusions. This first study on gut microbiota composition and resistome in human melioidosis showed extensive disruptions during hospitalization, with limited signs of restoration 6 months postinfection. Given the adverse outcomes linked with microbiome perturbations, limiting microbiota disruptions or using microbiota-restorative therapies (eg, butyrate-producing probiotics) may be beneficial.

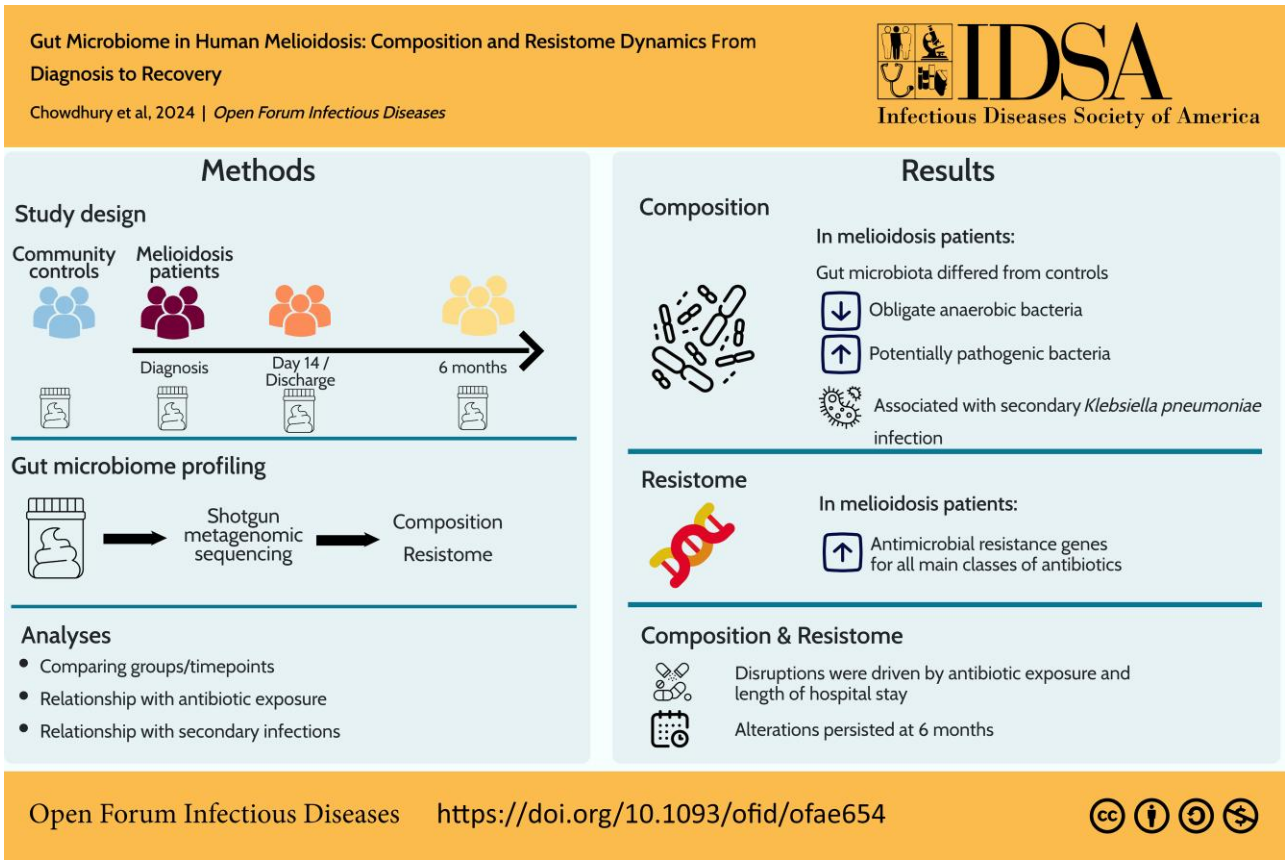
Received 10 July 2024; editorial decision 30 October 2024; accepted 1 November 2024; published online 5 November 2024

Correspondence: Chiranjay Mukhopadhyay, MD, PhD, Department of Microbiology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal 576104, Karnataka, India (chiranjay.m@manipal.edu).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofae654

Graphical Abstract



This graphical abstract is also available at Tidbit: https://tidbitapp.io/tidbits/microbial-resilience-in-human-melioidosis-understanding-gut-microbiome-and-resistome-dynamics-in-diagnosis-to-recovery-ffba86d1-98ab-4a6c-8340-37ca243314da

Keywords. AMR genes; gut microbiome; India; melioidosis; resistome.

Melioidosis, caused by *Burkholderia pseudomallei*, is a potentially fatal infectious disease with a significant global impact, endemic in Southeast Asia and Northern Australia, presenting diverse clinical manifestations and high mortality rates [1–4]. Melioidosis causes approximately 89 000 deaths per year globally [2], 30 000 of which are in India [3]. The global burden of melioidosis was estimated as 4.64 million disability-adjusted life years [5], highlighting the need for insights into melioidosis's pathophysiology and novel treatment strategies.

In recent years, animal studies have shown that intestinal microbiota can influence infectious disease outcomes. A healthy, undisrupted gut microbiota modulates systemic and mucosal immune responses, enhances the antimicrobial activity of neutrophils and alveolar macrophages, and prevents the translocation of potentially pathogenic gut bacteria into the systemic circulation and other distant organs [6–8]. Using a murine model of melioidosis, we demonstrated that the gut microbiota is disrupted during systemic infection by *B pseudomallei*, with a strong increase in Proteobacteria. In turn, perturbation of commensal microbiota (by antibiotic treatment) resulted in enhanced early dissemination of *B pseudomallei* and an increase in systemic proinflammatory cytokines [9], similar to other systemic infections [6–8]. Together, this suggests a protective role of the gut microbiota against various infectious diseases, including melioidosis, in mice. However, mice and human microbiota substantially differ and animal models might overstate the impact of disrupted gut microbiota on immune responses [10, 11], underscoring the necessity of translation of preclinical evidence to humans. Currently, there remains a substantial knowledge gap regarding the relationship between melioidosis and the human gut microbiome.

In systemic infections, studies from high-income countries have described gut microbiota alterations, characterized by a loss of obligate anaerobic commensals (eg, Lachnospiraceae and *Prevotella*) and an increase in potentially pathogenic bacterial species (eg, *Enterococcus*, *Klebsiella*) [6, 9, 12]. Such pathogen overgrowth correlates with an increased risk of secondary bloodstream infections with these organisms [13, 14]. Yet, studies describing gut microbiota during melioidosis—or any extraintestinal infections specifically in India—are lacking, whereas geographical and ethnic variation influences gut microbiota composition.

Treatment of melioidosis involves extensive antimicrobial therapy: an initial intensive phase (10–14 days) with ceftazidime or

meropenem, with or without trimethoprim-sulfamethoxazole (co-trimoxazole; if neurological, cutaneous, bone, joint, or prostatic manifestations). This is followed by an eradication phase of oral co-trimoxazole (3–6 months) [1, 15]. Given the important and long-lasting effects of antibiotics on gut microbiota, these treatments may further distort the microbiome, potentially increasing the risk of secondary infections and other adverse outcomes [16]. Extensive antimicrobial therapy may increase antimicrobial resistance (AMR) gene abundance in the gut, collectively called the "resistome." For example, a 5-day course of oral antibiotic treatment in healthy volunteers increased resistance genes, which persisted for 180 days [17]. In addition, antibiotic-induced gut microbiota perturbations increase susceptibility to colonization with multidrug-resistant bacteria [18]. Although AMR is a major threat to human health [19], data on gut resistome alterations following prolonged antibiotic treatment for infections are limited.

Here, we performed a prospective, longitudinal observational cohort study to profile, for the first time, the composition and resistome of gut microbiota during acute *B pseudomallei* infection and its recovery at 6 months postinfection. We hypothesized that gut microbiota will be distorted in melioidosis patients compared to community controls and we postulate that an increased prevalence of intestinal AMR genes persevere following cessation of treatment.

MATERIALS AND METHODS

Study Design

A prospective observational study at Kasturba Hospital (Manipal, India), from June 2020 to December 2022, enrolled adult patients (≥18 years) with culture-proven melioidosis. Community controls comprised patients' family members or neighbors of comparable age without recent infection, hospitalization, or antibiotic exposure. Ethical approval was obtained from the Kasturba Medical College and Kasturba Hospital Ethics Committee (reference number 77/2020) along with the informed consent of all study participants. The study design details and clinical data recording are provided in Supplementary Material 1.

Fecal Sample Collection and Processing

In patients with melioidosis, fecal samples were obtained on the day of diagnosis, 14 days thereafter, or the day of discharge (whichever occurred first), and after 6 months. For controls, a single fecal sample was collected. Fecal samples were collected in sterile containers and immediately stored at −80°C. DNA extraction was performed using the QIAamp DNA Extraction Power stool kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Microbiota Sequencing and Analysis

Details on gut microbiota sequencing, preprocessing, and analysis have been previously described elsewhere [20] and are detailed in the Supplementary Material 1. In brief, gut microbiotas were characterized using shotgun metagenomic sequencing of fecal samples on an Illumina Novaseq X platform (Illumina, San Diego, California). Raw sequence reads were quality-trimmed, human-decontaminated, and subsequently mapped to the GTDBr207 database [21, 22]. An average of 14.3 ± 5.5 million clean reads per sample were mapped. For the assembly of metagenome-assembled genomes (MAGs), quality-controlled paired-end reads were assembled individually with metaSPAdes (v3.15.5) [23]. Unmapped reads were then assembled with MEGAHIT (v1.2.4) [24]. metaWRAP (v1.0) workflows were used for the binning and refinement process, which were quality controlled by CheckM2 (v1.0.1) and GUNC (v1.0.5) [25–27]. To evaluate the presence of AMR genes, we used ABRicate with the National Center for Biotechnology Information database [28].

Statistical Analysis

Our statistical methods are detailed in Supplementary Material 1. Differences in microbiota composition and the contribution of clinical characteristics (eg, age, comorbidities, and antibiotic exposure) to interindividual microbiota differences were assessed using permutational multivariate analysis of variance (β-diversity using Bray-Curtis dissimilarities, 9999 permutations) [29, 30]. Differentially abundant bacteria between groups and timepoints were identified by MaAsLin2 [31]. Gut microbiota diversity was calculated using the Shannon diversity index on the species level. As previously described and validated [32–34], 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 [35]. Similar to previous work [36], overall resistome profiles were assessed by dimensionality reduction of the 216 unique AMR genes using Bray-Curtis dissimilarities. Associations between the abundance of butyrate-producing bacteria or number of AMR genes (grouped per class of antibiotics) and clinical variables (antibiotic treatment prior to sample collection, total number of days of antibiotic therapy, and length of hospital stay) were assessed by Pearson correlations (continuous variables) or Hedges' g (categorical variables). In addition, we compared the prevalence of each AMR gene between participants with and without meropenem exposure (Supplementary Material). No normalization of microbiota composition data was performed, whereas the number of AMR genes was normalized per million mapped bacterial reads.

RESULTS

Study Cohort

We obtained 70 fecal samples from 28 patients with culture-confirmed melioidosis and 16 community controls, which were successfully sequenced without significant batch effects

(Supplementary Figure 1). The clinical characteristics of the study participants are summarized in Table 1. Patients were predominantly male (96.4%) with a median age of 46.5 years (interquartile range [IQR], 42.0–57.3 years). The most prevalent comorbidity of melioidosis patients was diabetes (85.7%), followed by hypertension (42.9%) and renal dysfunction (21.4%). Melioidosis was disseminated in 15 patients (53.5%), while others had localized infection (46.4%). Blood (53.5%), deep-seated abscess (28.5%), and the lungs (17.8%) were the primary sites of infection. All patients received antibiotic treatment (Supplementary Figure 2); during hospital admission, patients with disseminated disease were mostly treated with ceftazidime (80.0%), co-trimoxazole (73.3%), and meropenem (60.0%). Co-trimoxazole (76.9%), meropenem (61.5%), and ceftazidime (30.8%) were also the most used antibiotics during hospitalization in patients with localized disease. During the eradication phase, 86.7% of patients with disseminated disease (n = 13) and 92.3% with localized disease (n = 12) received co-trimoxazole.

Table 1. Clinical Characteristics of the Study Population

Characteristic	Melioidosis (n = 28)	Community Controls (n = 16)	*P* Value
Age, y, median (IQR)	46.5 (42.0–57.3)	41.0 (35.3–45.0)	.071
Male sex	27 (96.4)	9 (56.2)	.004
BMI, kg/m^2, median (IQR)	27.0 (25.0–28.0)	25.0 (24.0–27.0)	.311
Alcoholism	17 (60.7)	6 (37.5)	.242
Comorbidities			
Any comorbidity	24 (85.7)	6 (37.5)	.003
Type 2 diabetes	24 (85.7)	6 (37.5)	.003
Diabetes duration			<.001
>5 y	4 (16.7)	1 (6.2)	
3–5 y	7 (29.2)	2 (12.5)	
1–2 y	11 (45.8)	3 (18.8	
Newly diagnosed	2 (8.3)	0 (0.0)	
HbA1c, %, median (IQR)	8.8 (7.7–10.6)	…	
Hypertension	12 (42.9)	…	
Chronic kidney disease	1 (3.6)	…	
Chronic liver disease	3 (10.7)	…	
COPD	1 (3.6)	…	
Clinical signs			
Fever	22 (78.6)	…	
Dyspnea	5 (17.9)	…	
Cough	8 (28.6)	…	
Neurological symptoms	4 (14.3)	…	
Primary site of infection (site of culture)			
Blood	15 (53.5)	…	
Deep-seated abscess[a]	8 (28.5)	…	
Lungs[b]	5 (17.8)	…	
Severity of disease			
SOFA score at admission, median (IQR)	1.00 (0.00–2.25)	…	
Disseminated disease	15 (53.5)	…	
Outcome			
Length of hospital stay, days, median (IQR)	15.5 (9.5–21.0)	…	
ICU admission	13 (46.4)	…	

Data are presented as No. of participants (%) unless otherwise indicated.

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; HbA1c, glycated hemoglobin; ICU, intensive care unit; IQR, interquartile range; SOFA, Sequential Organ Failure Assessment.

[a] *Burkholderia pseudomallei* was cultured from the material of the spleen (n = 3), liver (n = 3), scalp (n = 1), and shoulder abscesses (n = 1).

[b] *Burkholderia pseudomallei* was cultured from an endotracheal aspirate or bronchoalveolar lavage fluid.

The Gut Microbiota Is Persistently Altered During Acute Melioidosis and Recovery

Melioidosis was associated with profound alterations in the composition of the intestinal microbiota. When visualizing microbiota composition by principal coordinates analysis, considerable variation, and some overlap, between melioidosis patients and community controls was observed (Figure 1*A*). Yet, a detectable separation between community controls and melioidosis patients existed at all 3 timepoints (diagnosis vs controls: $P = .025$; day 14 vs controls: $P = .0003$; 6 months vs controls: $P = .0044$), implying differences in gut microbiota composition. In patients at diagnosis and controls, the presence of melioidosis was the strongest determinant of interindividual dissimilarities in gut microbiota composition compared to other clinical factors (age, sex, body mass index, and comorbidities) (Figure 1*B*). Gut microbiota α-diversity did not differ between patients and controls (Figure 1*C*). Microbiota of melioidosis patients had lower relative abundances of butyrate-producing bacteria, which showed limited signs of recovery at 6 months postdiagnosis (Figure 1*D*). In addition, decreased abundances of obligately anaerobic Firmicutes (eg, *Agathobacter*, *Faecalibacterium*) and *Prevotella* were observed in melioidosis cases at diagnosis and day 14 when compared to controls, whereas the prevalence and abundance of potentially pathogenic bacteria (eg, *Klebsiella pneumoniae*, *Enterococcus faecium*) profoundly increased during melioidosis (Figure 1*E*–*H*). Although such pathobionts were no longer significantly enriched at 6 months, these patients still had lower relative abundances of multiple—potentially beneficial—obligate anaerobes (Figure 1*I*), suggesting incomplete gut microbiota recovery. At diagnosis, the differences between patients and controls were not merely a consequence of antibiotic exposure, as the gut microbiota from patients not yet exposed to antibiotics at sample collection (n = 9 [34.6%]) also differed from that of controls (Supplementary Figure 3). Similarly, the difference in composition between controls and patients at diagnosis remained significant when we corrected for antibiotic exposure prior to sample collection in a multivariable model ($P = .013$). We thus concluded that the gut microbiota of melioidosis patients differed from community controls, with an increase in pathobionts and a long-lasting decrease in obligately anaerobic, butyrate-producing bacteria.

The Gut Resistome Is Persistently Altered During Acute Melioidosis and Recovery

Next, we investigated the relationship between infection with *B pseudomallei* (and its associated treatments) and the gut

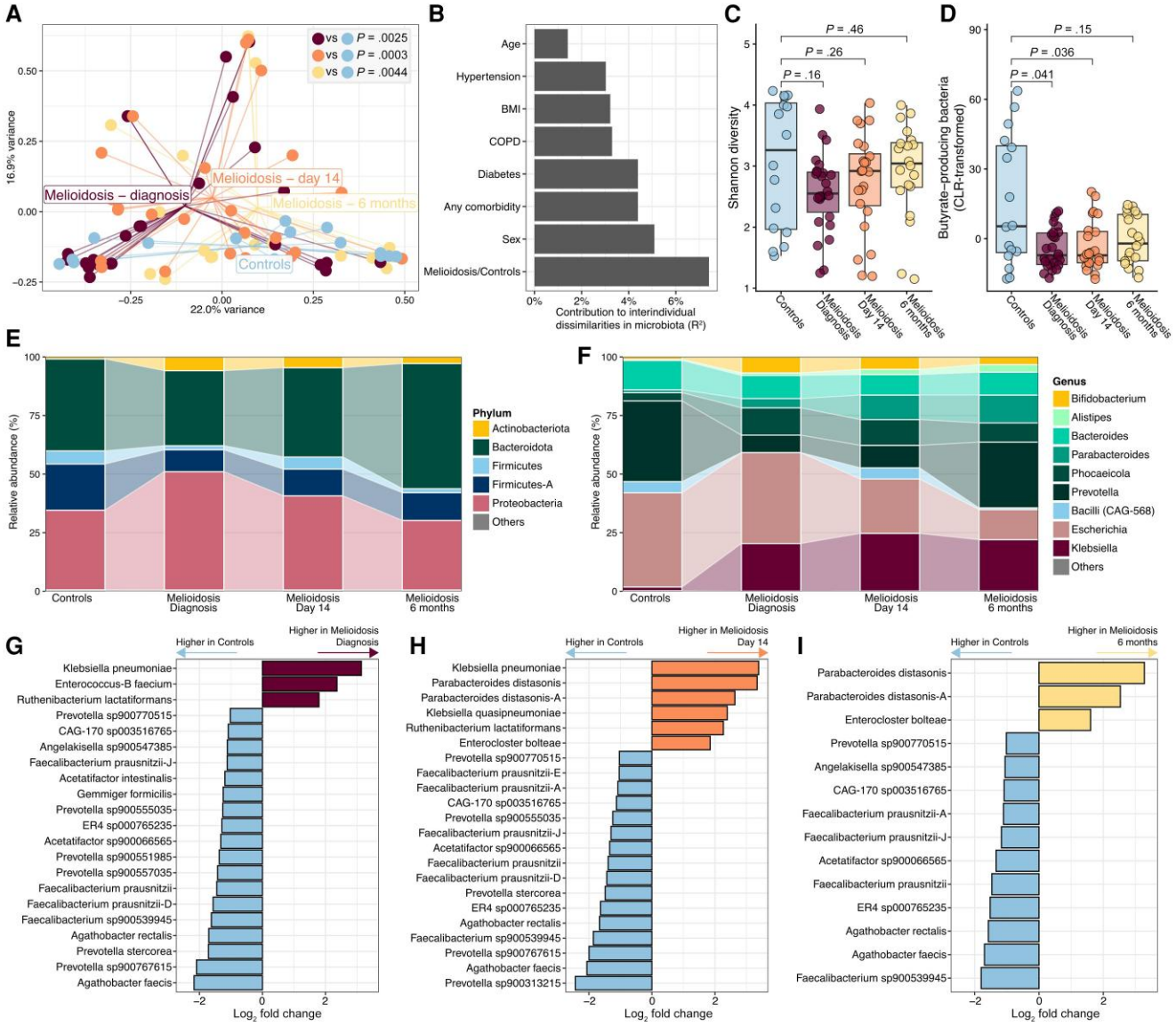


Figure 1. The gut microbiota is persistently altered during acute melioidosis and recovery. *A.* Gut microbiota composition (β-diversity) differed between patients with melioidosis (at diagnosis [n = 26], 14 days [n = 24], and 6 months [n = 20]) and community controls (n = 16). The significance of differences in community composition between these groups is determined using permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis dissimilarities. *B.* In patients at diagnosis and community controls, the presence of melioidosis (ie, patients vs controls) was the strongest determinant of interindividual dissimilarities (R^2) in gut microbiota composition when compared to other potential factors determining microbiota composition, determined by PERMANOVA with Bray-Curtis dissimilarities. *C.* Melioidosis patients and controls did not significantly differ in their gut microbiota diversity (Shannon diversity at species level). *D.* Patients at diagnosis and day 14 had lower relative abundances of butyrate-producing bacteria. Significance was assessed using the Wilcoxon rank-sum test. *E* and *F.* Bar plots show mean relative abundances of the main bacterial phyla (*E*) and genera (*F*) among patients with melioidosis and community controls. Different shades of the colors assigned to the 5 main phyla are used for genera within that phylum (eg, genera within the phylum Bacteroides are in green). *G.* Differences in microbiota composition were driven by higher relative abundances of *Klebsiella pneumoniae* (among others) and lower relative abundances of obligate anaerobic bacteria (eg, *Agathobacter faecis*, *Faecalibacterium prausnitzii*) in patients at diagnosis and community controls, as identified by MaAsLin2. *H.* Similar bacteria were identified when comparing patients at day 14 and community controls. *I.* At 6 months after melioidosis diagnosis, patients displayed higher levels of *Parabacteroides distasonis* and a reduction in obligate anaerobes. Abbreviations: BMI, body mass index; CLR, centered log-ratio; COPD, chronic obstructive pulmonary disease.

resistome. The total number of detected fecal AMR-associated genes was higher in melioidosis patients at all 3 timepoints (median, 2.7 genes per million mapped bacterial reads [IQR, 1.7–3.6]) compared to community controls (median, 1.0 [IQR, 0.8–1.7]) (Figure 2*A*). Overall resistome profiles showed a clear separation of patients (all timepoints) and controls, which was confirmed statistically via permutation testing (diagnosis vs controls: $P = .0003$; day 14 vs controls: $P = .0005$; 6 months vs controls: $P = .0063$; Figure 2*B*). Melioidosis samples from different timepoints overlapped and did not significantly differ.

In other words, intestinal AMR-associated genes are increased and exhibit an altered profile during acute melioidosis that persists during the recovery.

AMR genes providing resistance against multiple classes of antibiotics were detected in all patients and controls. β-Lactam (median, 0.43 genes per million mapped bacterial reads [IQR, 0.29–0.63]) and tetracycline (median, 0.43 [IQR, 0.24–0.58]) resistance genes were the most abundant, followed by macrolides (median, 0.30 [IQR, 0.20–0.47]; Figure 2*C*). In patients with melioidosis, the abundance of fecal resistance genes was increased for all main classes of antibiotics: aminoglycosides, β-lactams, carbapenems, chloramphenicol, macrolides, sulfonamide, quinolones, streptomycin, tetracyclines, and trimethoprim (Figure 2*D*). These conclusions were not affected by the normalization of the data (Supplementary Figure 4). We found no clear differences in the gut resistome between patients at diagnosis and following recovery, suggesting that the gut resistome is persistently altered after acute melioidosis.

Gut Microbiota Composition Following Recovery and the Resistome Are Linked With Antibiotic Exposure

Aligning with hospital recommendations on quantifying their use [32], we assessed the impact of the number of days of antibiotic therapy and the 4 most commonly used antibiotics in our cohort: co-trimoxazole, cephalosporins, meropenem, and β-lactams with a β-lactamase inhibitor (amoxicillin-clavulanate, ticarcillin-clavulanate, cefoperazone-sulbactam, and piperacillin-sulbactam). Exposure to cephalosporins ($R^2 = 0.10$) and co-trimoxazole ($R^2 = 0.093$), the number of days of antibiotic therapy ($R^2 = 0.094$), and the total length of hospital stay ($R^2 = 0.088$) were the most important contributors to interindividual dissimilarities in the microbiota composition of patients at 6 months (Figure 3*A*). Considering that demographics, diet, comorbidities, medication, and socioeconomic factors often together explain similar variance in microbiota composition ($R^2 = 0.09$–0.12) in large cohort studies, the effects of these variables were relatively large. Patients at 6 months exposed to co-trimoxazole and cephalosporins displayed reduced abundances of multiple *Prevotella* species and the strict anaerobe *Anaeromassilibacillus stercoravium*, respectively (Figure 3*B*). Several *Bacteroides*, *Parabacteroides*, and *Alistipes* species were increased in patients with a higher number of days of antibiotic therapy and longer length of hospital stay (Figure 3*B*). Moreover, in line with its clinical activity, meropenem was associated with the strongest decrease in obligate anaerobic butyrate producers (Figure 3*C*).

The gut resistome was similarly associated with antibiotic exposure and hospital stay. A longer hospital stay was associated with an increase in AMR genes for all antibiotics, except aminoglycosides, and more extensive antibiotic treatment correlated with higher abundances of resistance genes against β-lactams, carbapenems, chloramphenicol, macrolides, and tetracyclines (Figure 3*D*). Specifically, exposure to meropenem before sample collection was associated with a sharp increase in resistance against multiple classes of antibiotics, while the effects of other antibiotics on resistome were less pronounced (Figure 3*E*). Correcting for the total length of hospital stay in a multivariable linear model yielded similar associations between the gut resistome and meropenem exposure (Supplementary Figure 5). In participants exposed to meropenem, 11 AMR-associated genes were detected in a higher proportion (ie, prevalence) compared to those without meropenem usage (Figure 3*F*). Of these, 2 (*vanY-A* and *qnrB7*) were recently classified as public health threats of the highest category [37, 38]. Together, this shows that the length of hospital stays and extent of antibiotic treatment (specifically the usage of meropenem) drive differences in microbiota composition and resistome following recovery from melioidosis.

Microbiota Alterations Precede Nosocomial Systemic Infection With *K pneumoniae*

Given the higher abundances of *K pneumoniae* during melioidosis and because *K pneumoniae* was the most common cause of secondary infection (8 of 28 patients, coinfection in 75% of cases; Supplementary Figure 6), we explored the relationship between fecal *K pneumoniae* and secondary *K pneumoniae* infections. Clinical characteristics of patients with secondary *K pneumoniae* infections are summarized in Supplementary Table 1. The gut microbiota composition of melioidosis patients with secondary *K pneumoniae* differed from those without secondary *K pneumoniae* infection (Figure 4*A*). These differences were statistically significant when comparing all samples taken during hospitalization of these groups ($P = .0038$), but also when limited to only fecal samples collected prior to the first positive culture with *K pneumoniae* (ie, at melioidosis diagnosis; $P = .044$). Although the relative abundance of *K pneumoniae* peaked around diagnosis (Figure 4*B*), we did not find a significant difference in *K pneumoniae* levels between patients with and those without secondary *K pneumoniae* infection ($P = .44$; Figure 4*C*). Gut microbiota diversity and the abundance of butyrate-producing bacteria did not significantly differ between patients with and those without secondary *K pneumoniae* infection (Supplementary Figure 7). Finally, we compared the antimicrobial susceptibility of the clinical isolates of *K pneumoniae* causing the secondary infection (ie, retrieved from positive cultures) with the presence of AMR genes in *K pneumoniae* from the gut metagenome. Gut metagenome bins (MAGs) taxonomically classified as *K pneumoniae* were identified in 4 patients (of 8 with secondary *K pneumoniae* infection; 1–3 MAGs per patient). In all of these MAGs, resistance genes for quinolones and cephalosporins (ie, β-lactam) were identified, whereas AMR genes for aminoglycosides (5/9 MAGs [55.6%]), co-trimoxazole

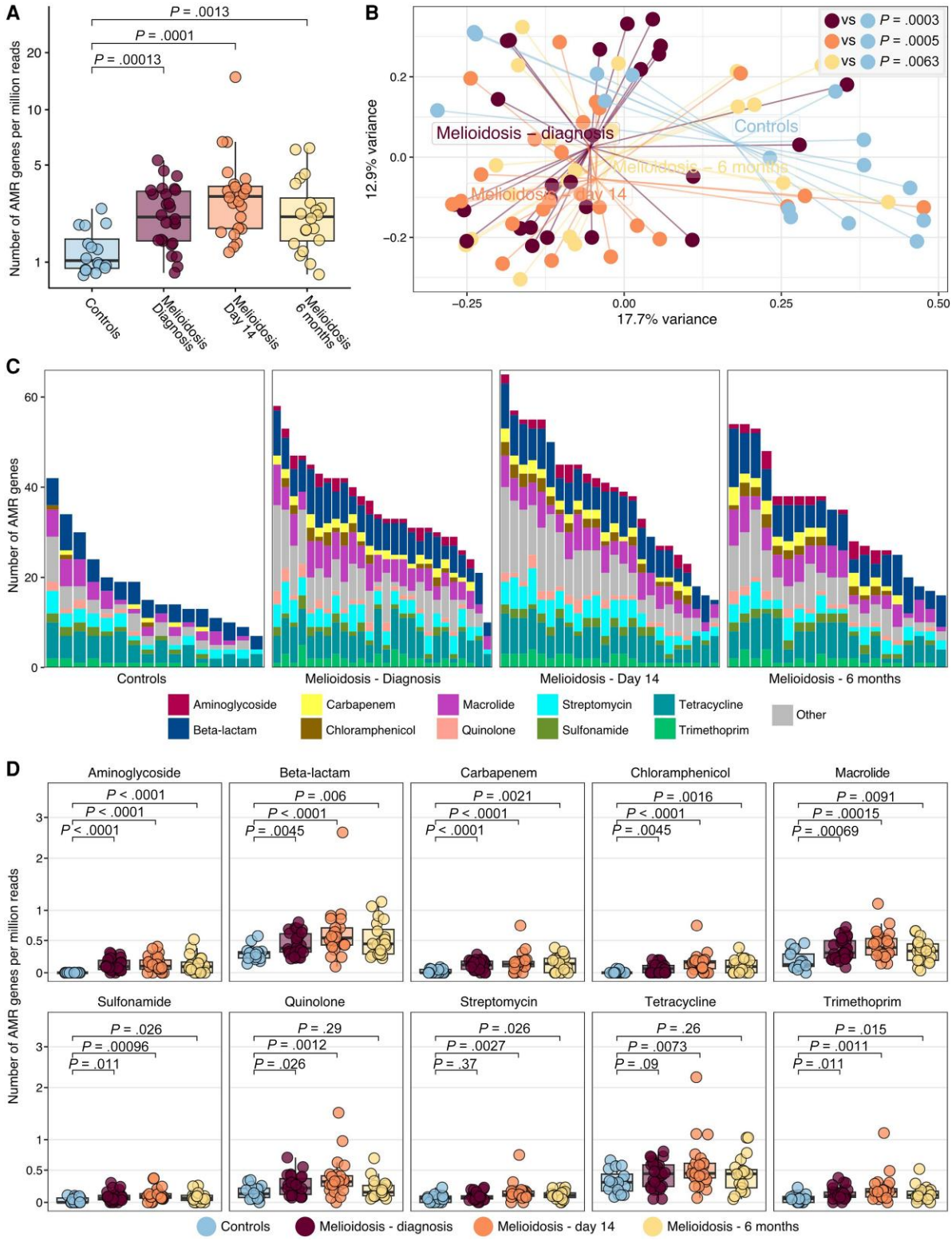


Figure 2. The gut resistome is persistently altered during acute melioidosis and recovery. *A.* A larger number of antimicrobial resistance (AMR) genes (normalized per million mapped bacterial reads) was detected in melioidosis patients at diagnosis (n = 26), 14 days after diagnosis (n = 24), and 6 months (n = 20), compared to community controls (n = 16; Wilcoxon rank-sum test). *B.* Principal coordinates analysis of AMR genes (Bray-Curtis dissimilarities) showed clear separation of the resistome of melioidosis patients and controls. Permutational multivariate analysis of variance was used to assess the significance of differences in resistome composition. *C.* Bar plots depicting the number of AMR genes detected per group. Each bar represents 1 sample and the associated classes of antibiotics are indicated with colors. *D.* For all of the main antibiotic classes, a larger number of AMR genes per class was detected in patients with melioidosis compared to community controls (Wilcoxon rank-sum test).

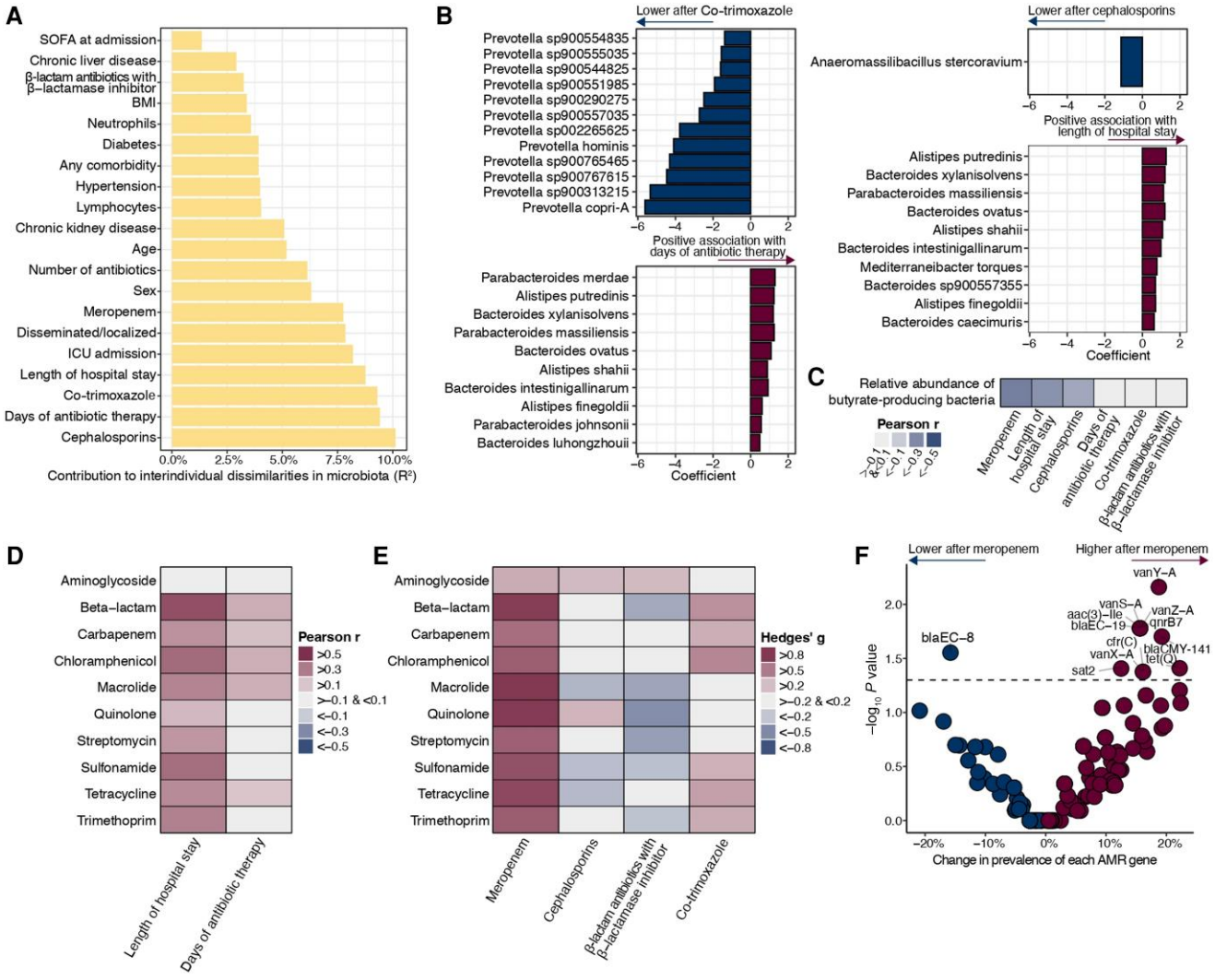


Figure 3. Contribution of clinical variables to microbiota composition and resistome. *A.* Impact of clinical variables on interindividual dissimilarities (R^2) in gut microbiota composition of melioidosis patients at 6 months, determined by permutational multivariate analysis of variance with Bray-Curtis dissimilarities. *B.* A MaAsLin2 model identified differentially abundant species in the gut microbiota of melioidosis patients at 6 months who were treated with co-trimoxazole (compared to patients without co-trimoxazole treatment) or with cephalosporins (compared to no cephalosporins). Furthermore, MaAsLin2 identified species associated with the total number of days of antibiotic therapy and with the length of hospital stay. *C.* Pearson correlations between the relative abundance of butyrate-producing bacteria and the length of hospital stay or exposure to antibiotics. *D.* Pearson correlations between the number of antimicrobial resistance (AMR) genes per antibiotic class and the length of hospital stay or days of antibiotic therapy. *E.* Hedges' g effect size for the relationship between the number of AMR genes per antibiotic class (y-axis) and exposure to 4 antibiotics prior to sample collection (x-axis). *F.* Volcano plot comparing the prevalence of each AMR-associated gene (ie, the proportion of samples in which an individual gene was detected) between those with and without prior meropenem exposure. Labeled AMR genes passed the threshold of $P < .05$. Abbreviations: AMR, antimicrobial resistance; BMI, body mass index; ICU, intensive care unit; SOFA, Sequential Organ Failure Assessment.

(4/9 [44.4%]), and carbapenems (4/9 [44.4%]) were less common (Supplementary Figure 8). In these 4 patients, clinical *K pneumoniae* isolates were resistant to cephalosporins, quinolones, and co-trimoxazole, while the isolate of patient 4 also was resistant to aminoglycosides and carbapenems (Supplementary Figure 8). Although some overlap in resistance patterns between the clinical isolate and gut metagenome bins was common, none of the MAGs fully matched the resistance pattern of the clinical isolate (Supplementary Figure 8). Together, these exploratory analyses show that gut microbiota alterations are linked with nosocomial infection with multidrug-resistant *K pneumoniae* in patients with melioidosis.

DISCUSSION

In this longitudinal, observational cohort study, we showed that the gut microbiota of melioidosis patients differ from community controls, with a decrease in obligate anaerobic bacteria during melioidosis and an increase of potentially pathogenic

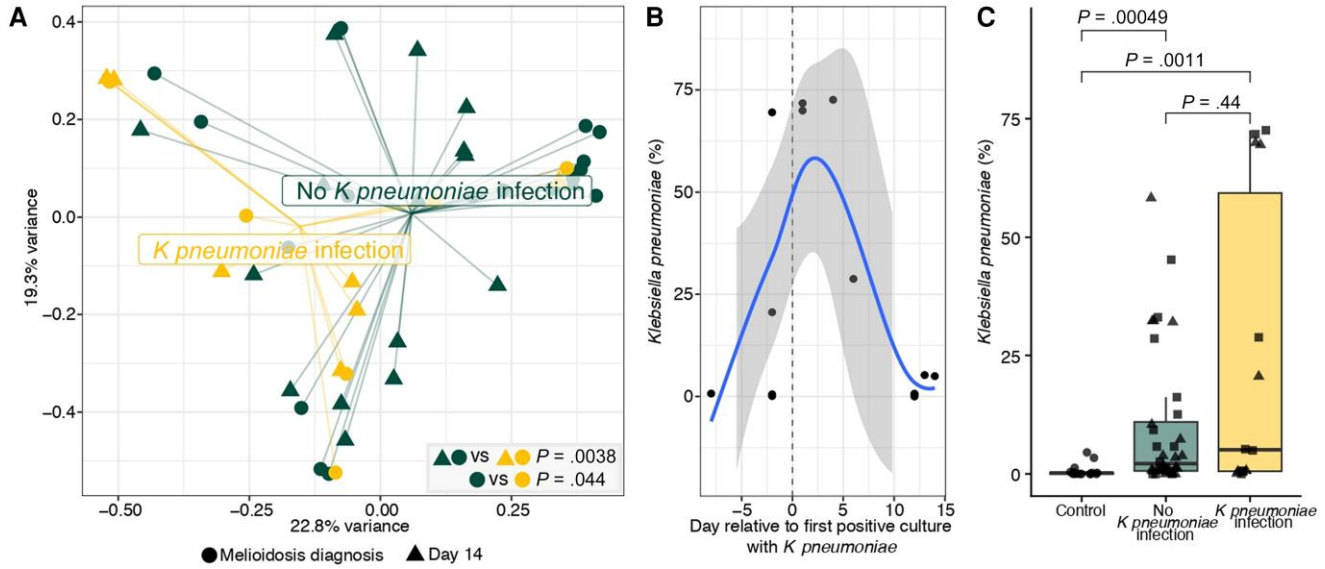


Figure 4. The gut microbiota is associated with secondary *Klebsiella pneumoniae* infection. *A.* Gut microbiota composition (β-diversity) differed between melioidosis patients with (n = 8) and without (n = 20) a secondary infection by *K pneumoniae*. Samples collected during the hospital stay (at diagnosis of melioidosis or day 14, indicated with shapes) were compared. The significance of differences in community composition between groups was determined using permutational multivariate analysis of variance with Bray-Curtis dissimilarities. *B.* In melioidosis patients with a secondary *K pneumoniae* infection, the relative abundance of *K pneumoniae* in the gut microbiome peaked around the first positive culture with *K pneumoniae*. The solid line represents the dynamic trend, with the shaded area indicating the 95% confidence interval. *C.* Melioidosis patients with and those without secondary *K pneumoniae* infection did not significantly differ in their fecal relative abundance of *K pneumoniae*. Yet, in both, abundances were higher than in community controls (Wilcoxon rank-sum test).

bacteria, such as *K pneumoniae*, which may be linked with an increased risk of secondary *K pneumoniae* infections. The gut microbiota of melioidosis patients showed limited signs of recovery following hospital discharge, with particularly a long-lasting decrease in butyrate producers. In addition, the abundance of AMR genes conferring resistance against all main classes of antibiotics was persistently increased in the gut microbiome of melioidosis patients. To the best of our knowledge, this is the first study describing gut microbiota alterations during and following melioidosis, and among the first to describe the long-term impact of systemic infection and extensive antibiotic therapy on the gut resistome. These findings translate evidence from animal models to humans and—given the adverse outcomes linked with microbiome perturbations—underscore the potential benefit of limiting microbiota disruptions or administering microbiota-restorative therapies.

We and others have shown that gut microbiota alterations are common during infectious diseases [33, 37]. Here, we expand these findings to human melioidosis: Gut microbiota composition during melioidosis (all 3 timepoints) differed from that of healthy controls from the same community. Similar to other infectious diseases, we found a decrease in obligate anaerobic butyrate-producing bacteria (eg, *Faecalibacterium* and *Agathobacter*) and an increase in potential pathogens (eg, *K pneumoniae*). The presence of melioidosis was a key factor in determining microbiota composition, over other variables. Murine studies showed that systemic infections can induce such gut microbiota changes, which subsequently may influence disease severity. For example, we demonstrated that systemic *B pseudomallei* infection induces an increase in Proteobacteria, and mice with disrupted gut microbiota had enhanced systemic inflammation and early dissemination during experimental melioidosis [9]. Long-term changes in our cohort may be a consequence of the prolonged and intensive use of antibiotics, which could select resistant bacteria and deplete obligate anaerobes that provide colonization resistance through nutrient competition, immunomodulation, and maintenance of the anaerobic environment [38–40]. Notably, antibiotic-induced microbiota perturbations may have negative long-term health consequences: Presumed microbiota disruptions (eg, more extensive antibiotic treatment) have been linked with a higher risk of readmission with severe sepsis [20, 41]. Yet, it is unclear whether this represents a treatable trait and if microbiota-restorative therapies (most importantly, butyrate-producing probiotics) could improve long-term outcomes and reduce AMR gene prevalence in patients with melioidosis.

Although the microbiota alterations during melioidosis were largely similar to those reported in other systemic infections [8, 10], it remains unclear whether different bacterial infections have different effects on gut microbiota, or if effects are similar for all pathogens. Future studies may identify pathogen-specific microbiome alterations, potentially through metabolomic and

resistome profiling. Furthermore, gut microbiota alterations in melioidosis patients overlapped with changes described in diabetes (eg, an increase in opportunistic pathogens such as *Escherichia coli* and *Enterococcus faecalis*, and reduced abundance of butyrate-producing bacteria such as *Faecalibacterium prausnitzii*) [42], which is the most common comorbidity in patients with melioidosis [4]. Whether these microbiota alterations are linked with both diabetes and melioidosis through common immunomodulatory pathways, or a more complex relationship between gut microbiota, diabetes, and melioidosis exists, remains to be elucidated.

Previous studies found that depletion of gut anaerobes and simultaneous domination by potential pathogens, like Proteobacteria or Enterobacteriaceae, preceded nosocomial infections [16, 17]. Since *K pneumoniae* was the most strongly enriched bacterium in melioidosis patients and the most common cause of nosocomial infection in our cohort, we explored the relationship between intestinal *K pneumoniae* and secondary infections. Intestinal abundances of *K pneumoniae* peaked around the time that the secondary *K pneumoniae* infection was diagnosed (ie, at positive culture), and gut microbiota composition of patients with secondary *K pneumoniae* infection differed from those without such an infection. This may suggest translocation of *K pneumoniae* from the intestine to the blood and other organs, although we found only partial concordance between clinical antimicrobial susceptibility and the presence of AMR genes in gut metagenome data of *K pneumoniae*. Arguing against such direct translocation could also be caused by technical limitations of resistome annotations. Similar analyses in larger clinical cohorts will be an important next step to further uncover the relationship between intestinal overgrowth of pathogens and secondary infections.

Although multiple studies described gut microbial composition changes during systemic infections, limited data exist on resistome alterations. Here, through our longitudinal design and untargeted resistome analyses, we found a clear separation in resistome profiles between melioidosis patients and controls, which remained at 6 months postdiagnosis. The length of hospital stay and extent of antibiotic treatment were the strongest determinants of interindividual dissimilarities in microbiota composition and resistome following recovery. These findings align with other diseases requiring long-term antibiotic treatment, such as tuberculosis [43]. Specifically, meropenem exposure was linked with an increase in 11 AMR genes, including *vanY-A* and *qnrB7*, classified as a public health threat [44, 45], and extending beyond carbapenems to other antibiotic classes. This meropenem-associated gut resistome expansion may be considered in clinical decision-making between ceftazidime or carbapenems during melioidosis treatment as limited data on the inferiority of ceftazidime in the absence of septic shock are available [46]. Moreover, accumulating evidence shows that shorter antibiotic therapy regimens may be safe for many infections [47, 48], whereas recent observational and translational studies linked the use of anti-anaerobic antibiotics to higher mortality in patients with severe infections when compared to antibiotics with less impact on anaerobic gut microbiota [49, 50]. Moreover, a recent multicenter trial in 658 patients with melioidosis found that a 12-week regimen of co-trimoxazole may be preferable for the eradication treatment of melioidosis compared to 20 weeks [51]. Given the observed gut microbiota and resistome disruptions during melioidosis, with potential health consequences, a key message from our study is the call for additional trials examining possibilities to safely shorten antibiotic courses for melioidosis.

Strengths of this study include the longitudinal follow-up, well-annotated cohort, metagenomic sequencing microbiome analyses, and the translation of preclinical evidence to humans. We provide the first description of gut microbiota in melioidosis patients and the first study in any extraintestinal infection from India. Although existing research from high-income countries has described gut microbiota alterations in systemic infections, there remains a notable gap in understanding these dynamics within the context of low- and middle-income settings, while geographical and ethnic variations have important effects on gut microbiota. This study has several limitations. First, the limited number of patients may restrict the generalizability of our findings to other melioidosis patients and impedes our ability to draw strong conclusions, especially regarding the association between the microbiota and secondary infections. Second, our study's observational design constrains its capacity to establish direct cause-and-effect relationships. Yet, our findings are consistent with murine studies showing the effects of *B pseudomallei* infection on the gut microbiota [9]. Third, this study utilized DNA sequencing, limiting our ability to assess the expression of AMR genes over time.

In summary, this first study on gut microbiota composition and resistome in human melioidosis showed extensive disruptions during hospitalization, with limited signs of restoration 6 months postinfection. Given the adverse outcomes linked with microbiome perturbations, clinical trials are needed to assess whether administering microbiota-restorative therapies or limiting microbiota disruptions by reducing prolonged exposure to antimicrobials would improve clinical outcomes.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We are most grateful to all patients, their family members, and neighbors who participated in the study, and to all staff involved in data collection.

Author contributions. S. C. designed the study, performed sample and clinical data collection, interpreted the analysis, and wrote the manuscript. R. F. J. K. performed and interpreted the analysis and wrote the manuscript. B. W. H., C. D., and T. D. L. performed microbiome data preprocessing and interpreted the data. V. A. E. and V. K. E. assisted in writing and reviewing the manuscript. W. J. W. assisted with result interpretation and reviewing the manuscript. C. M. designed the study, assisted with the results, and assisted with writing and reviewing the manuscript. All authors approved the manuscript and agreed to submit it for publication.

Data availability. Sequence files and metadata for all samples used in this study are available in the Sequence Read Archive under accession number PRJNA1090271 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1090271?reviewer = 9e3dj25npjue8pcdkh9s80398 m). Original R scripts are available on GitHub (https://github.com/rfjkullberg/microbiota_human_melioidosis). Sample metadata and the processed microbiota abundance data are available as Supplementary Material 2 and 3, respectively. The clinical data of individual patients in this cohort may be accessible upon reasonable request from the corresponding author.

Ethics approval. Ethical approval was obtained from the Kasturba Medical College and Kasturba Hospital Ethics Committee (reference number 77/2020) along with the informed consent of all study participants.

Disclaimer. The funder had no role in study design, data collection and analysis, preparation of the manuscript, or the decision to publish.

Financial support. This study was supported by a grant from the Indian Council of Medical Research (ICMR), New Delhi, to C. M. (grant number 2020-1381).

Potential conflicts of interest. The authors declare no potential conflicts of interest.

References

1. Meumann EM, Limmathurotsakul D, Dunachie SJ, Wiersinga WJ, Currie BJ. *Burkholderia pseudomallei* and melioidosis. Nat Rev Microbiol **2024**; 22: 155–69.
2. Limmathurotsakul D, Golding N, Dance DA, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol **2016**; 1:1–5.
3. Mukhopadhyay C, Shaw T, Varghese G, Dance D. Melioidosis in South Asia (India, Nepal, Pakistan, Bhutan and Afghanistan). Trop Med Infect Dis **2018**; 3:51.
4. Wiersinga WJ, Virk HS, Torres AG, et al. Melioidosis. Nat Rev Dis Primers **2018**; 4:1–22.
5. Wiersinga WJ, Currie BJ, Peacock SJ. Melioidosis. N Engl J Med **2012**; 367: 1035–44.
6. Schuijt TJ, Lankelma JM, Scicluna BP, et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. Gut **2016**; 65:575–83.
7. Deshmukh HS, Liu Y, Menkiti OR, et al. The microbiota regulates neutrophil homeostasis and host resistance to *Escherichia coli* K1 sepsis in neonatal mice. Nat Med **2014**; 20:524–30.
8. Haak BW, Wiersinga WJ. The role of the gut microbiota in sepsis. Lancet Gastroenterol Hepatol **2017**; 2:135–43.
9. Lankelma JM, Birnie E, Weehuizen TAF, et al. The gut microbiota as a modulator of innate immunity during melioidosis. PLoS Negl Trop Dis **2017**; 11:e0005548.
10. Kullberg RFJ, Wiersinga WJ, Haak BW. Gut microbiota and sepsis: from pathogenesis to novel treatments. Curr Opin Gastroenterol **2021**; 37:578–85.
11. Dickson RP, Singer BH, Newstead MW, et al. Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. Nat Microbiol **2016**; 1:1–9.
12. Birnie E, Virk HS, Savelkoel J, et al. Global burden of melioidosis in 2015: a systematic review and data synthesis. Lancet Infect Dis **2019**; 19:892–902.
13. Walter J, Armet AM, Finlay BB, Shanahan F. Establishing or exaggerating causality for the gut microbiome: lessons from human microbiota–associated rodents. Cell **2020**; 180:221–32.
14. Park JC, Im SH. Of men in mice: the development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. Exp Mol Med **2020**; 52: 1383–96.
15. Sullivan RP, Marshall CS, Anstey NM, Ward L, Currie BJ. 2020 review and revision of the 2015 Darwin melioidosis treatment guideline; paradigm drift not shift. PLoS Negl Trop Dis **2020**; 14:1–13.
16. Taur Y, Xavier JB, Lipuma L, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis **2012**; 55:905–14.
17. Zhai B, Ola M, Rolling T, et al. High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. Nat Med **2020**; 26:59–64.
18. Freedberg DE, Zhou MJ, Cohen ME, et al. Pathogen colonization of the gastrointestinal microbiome at intensive care unit admission and risk for subsequent death or infection. Intensive Care Med **2018**; 44:1203–11.
19. Murray CJ, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet **2022**; 399:629–55.
20. Baggs J, Jernigan JA, Halpin AL, Epstein L, Hatfield KM, McDonald LC. Risk of subsequent sepsis within 90 days after a hospital stay by type of antibiotic exposure. Clin Infect Dis **2018**; 66:1004–12.
21. Anthony WE, Wang B, Sukhum KV, et al. Acute and persistent effects of commonly used antibiotics on the gut microbiome and resistome in healthy adults. Cell Rep **2022**; 39:110649.
22. Isles NS, Mu A, Kwong JC, Howden BP, Stinear TP. Gut microbiome signatures and host colonization with multidrug-resistant bacteria. Trends Microbiol **2022**; 30:853–65.
23. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. MetaSPAdes: a new versatile metagenomic assembler. Genome Res **2017**; 27:824–34.
24. Li D, Liu CM, Luo R, Sadakane K, Lam TW. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics **2015**; 31:1674–6.
25. Uritskiy GV, Diruggiero J, Taylor J. MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome **2018**; 6:1–13.
26. Chklovski A, Parks DH, Woodcroft BJ, Tyson GW. Checkm2: a rapid, scalable and accurate tool for assessing microbial genome quality using 1 machine learning 2 3 4. Nat Med **2023**; 20:1203–12.
27. Orakov A, Fullam A, Coelho LP, et al. GUNC: detection of chimerism and contamination in prokaryotic genomes. Genome Biol **2021**; 22:1–9.
28. Seemann T. ABRicate: mass screening of contigs for antimicrobial resistance or virulence genes. **2020**. Available at: https://github.com/tseemann/abricate (accessed on February 2, 2024).
29. Oksanen J, Simpson GL, Blanchet FG, et al. Vegan: community ecology package. **2022**. Available at: https://CRAN.R-project.org/package=vegan (accessed on January 14, 2024).
30. McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One **2013**; 8: e61217.
31. Mallick H, Rahnavard A, McIver LJ, et al. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol **2021**; 17:e1009442.
32. Haak BW, Littmann ER, Chaubard J-L, et al. Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. Blood **2018**; 131:2978–86.
33. Haak BW, De Jong HK, Kostidis S, et al. Altered patterns of compositional and functional disruption of the gut microbiota in typhoid fever and nontyphoidal febrile illness. Open Forum Infect Dis **2020**; 7:ofaa251.
34. Kullberg RFJ, Wikki I, Haak BW, et al. Association between butyrate-producing gut bacteria and the risk of infectious disease hospitalisation: results from two observational, population-based microbiome studies. Lancet Microbe **2024**; 5: 100864.
35. Vital M, Karch A, Pieper DH. Colonic butyrate-producing communities in humans: an overview using omics data. mSystem **2017**;2:10–128.
36. Ducarmon QR, Zwittink RD, Willems RPJ, et al. Gut colonisation by extended-spectrum β-lactamase-producing *Escherichia coli* and its association with the gut microbiome and metabolome in Dutch adults: a matched case-control study. Lancet Microbe **2022**; 3:e443–51.
37. Zhang AN, Gaston JM, Dai CL, et al. An omics-based framework for assessing the health risk of antimicrobial resistance genes. Nat Commun **2021**; 12: 4765.
38. Worby CJ, Sridhar S, Turbett SE, et al. Gut microbiome perturbation, antibiotic resistance, and *Escherichia coli* strain dynamics associated with international travel: a metagenomic analysis. Lancet Microbe **2023**; 4:e790–9.
39. Gu S, Chen Y, Wu Z, et al. Alterations of the gut microbiota in patients with COVID-19 or H1N1 influenza. Clin Infect Dis **2020**; 71:2669–78.
40. Pamer EG. Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. Science **2016**; 352:535–8.
41. Kim S, Covington A, Pamer EG. The intestinal microbiota: antibiotics, colonization resistance, and enteric pathogens. Immunol Rev **2017**; 279:90–105.
42. Litvak Y, Byndloss MX, Bäumler AJ. Colonocyte metabolism shapes the gut microbiota. Science **2018**; 362:eaat9076.
43. Prescott HC, Dickson RP, Rogers MAM, Langa KM, Iwashyna TJ. Hospitalization type and subsequent severe sepsis. Am J Respir Crit Care Med **2015**; 192:581–8.

44. Baars DP, Fondevila MF, Meijnikman AS, Nieuwdorp M. The central role of the gut microbiota in the pathophysiology and management of type 2 diabetes. Cell Host Microbe **2024**; 32:1280–300.
45. Vich Vila A, Collij V, Sanna S, et al. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. Nat Commun **2020**; 11:362.
46. Cheng AC, Fisher DA, Anstey NM, Stephens DP, Jacups SP, Currie BJ. Outcomes of patients with melioidosis treated with meropenem. Antimicrob Agents Chemother **2004**; 48:1763–5.
47. Royer S, Demerle KM, Dickson RP, Prescott HC. Shorter versus longer courses of antibiotics for infection in hospitalized patients: a systematic review and meta-analysis. J Hosp Med **2018**; 13:336–42.
48. Spellberg B, Rice LB. Duration of antibiotic therapy: shorter is better. Ann Intern Med **2019**; 171:210–1.
49. Chanderraj R, Baker JM, Kay SG, et al. In critically ill patients, anti-anaerobic antibiotics increase risk of adverse clinical outcomes. Eur Respir J **2023**; 61: 2200910.
50. Chanderraj R, Admon AJ, He Y, et al. Mortality of patients with sepsis administered piperacillin-tazobactam vs cefepime. JAMA Intern Med **2024**; 184:769–77.
51. Anunnatsiri S, Chaowagul W, Teparrukkul P, et al. A comparison between 12 versus 20 weeks of trimethoprim-sulfamethoxazole as oral eradication treatment for melioidosis: an open-label, pragmatic, multicenter, non-inferiority, randomized controlled trial. Clin Infect Dis **2021**; 73:E3627–33.