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Alterations of the Oral Microbiome and Cumulative Carbapenem Exposure Are Associated With *Stenotrophomonas maltophilia* Infection in Patients With Acute Myeloid Leukemia Receiving Chemotherapy

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(See the Editorial Commentary by Fredricks on pages 1514–6.)

Background. *Stenotrophomonas maltophilia* is increasingly common in patients with acute myeloid leukemia (AML). Little is known about factors that drive *S. maltophilia* infection. We evaluated the microbiome and cumulative antibiotic use as predictors of *S. maltophilia* infection in AML patients receiving remission induction chemotherapy (RIC).

Methods. Subanalysis of a prospective, observational cohort of patients with AML receiving RIC between September 2013 and August 2015 was performed. Fecal and oral microbiome samples collected from initiation of RIC until neutrophil recovery were assessed for the relative abundance of *Stenotrophomonas* via 16S rRNA gene quantitation. The primary outcome, microbiologically proven *S. maltophilia* infection, was analyzed using a time-varying Cox proportional hazards model.

Results. Of 90 included patients, 8 (9%) developed *S. maltophilia* infection (pneumonia, n = 6; skin–soft tissue, n = 2); 4/8 (50%) patients were bacteremic; and 7/8 (88%) patients with *S. maltophilia* infection had detectable levels of *Stenotrophomonas* vs 22/82 (27%) without infection ($P < .01$). An oral *Stenotrophomonas* relative abundance of 36% predicted infection (sensitivity, 96%; specificity, 93%). No association of *S. maltophilia* infection with fecal relative abundance was found. Cumulative meropenem exposure was associated with increased infection risk (hazard ratio, 1.17; 95% confidence interval, 1.01–1.35; $P = .03$).

Conclusions. Here, we identify the oral microbiome as a potential source for *S. maltophilia* infection and highlight cumulative carbapenem use as a risk factor for *S. maltophilia* in leukemia patients. These data suggest that real-time monitoring of the oral cavity might identify patients at risk for *S. maltophilia* infection.

Keywords. pneumonia; bacteremia; risk factors; colonization; meropenem.

Stenotrophomonas maltophilia is an intrinsically multidrug-resistant (MDR) gram-negative bacteria and the most frequently identified carbapenem-resistant gram-negative species in hospitalized patients with pneumonia [1, 2]. *Stenotrophomonas maltophilia* is increasingly identified in patients with cancer and is associated with high morbidity and mortality in this highly vulnerable population [2, 3]. Patients with acute myeloid leukemia (AML) are at particularly high risk for poor outcomes, with overall mortality in excess of 20% in patients with primary bacteremia and 60% for patients with pneumonia [4–7]. In its most devastating form, *S. maltophilia* infection manifests as hemorrhagic pneumonia with a case fatality rate approaching 100% [4]. As *S. maltophilia* is intrinsically resistant to the majority of antibiotics used to empirically treat febrile neutropenia in patients with AML and delayed appropriate antibiotic treatment is associated with increased mortality, identification of patients at risk for *S. maltophilia* infection is of paramount importance [8].

Due to intrinsic carbapenem resistance, prior carbapenem use appears to be the predominant risk factor for infection with *S. maltophilia*, and prior studies have identified carbapenem use, among other common factors such as prolonged hospital stay and intensive care unit admission, as a key risk factor [9–12]. Empiric carbapenem use is increasingly common in patients with AML due to rising rates of infections caused by extended-spectrum β-lactamase–producing organisms; therefore, an in-depth understanding of the risk–benefit profile of widespread carbapenem use is of high importance [13, 14].

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However, prior studies have largely evaluated carbapenem exposure as a dichotomous variable or in arbitrarily categorized numbers of days, preventing an understanding of how cumulative carbapenem exposure modifies risk for subsequent *S. maltophilia* infection [2].

Colonization with MDR organisms, detected through either traditional means or microbiome analysis, is clearly linked to subsequent infection in patients with hematologic malignancies [15–17]. Indeed, a recent study performed in hematopoietic stem cell transplant recipients identified oral colonization with *S. maltophilia* as being significantly associated with *S. maltophilia* infection [18]. That study did not, however, integrate antimicrobial exposure or allow for a quantitative assessment of *S. maltophilia* burden in relation to infection. Thus, we sought to characterize cumulative antibiotic exposure and the relative abundance of *S. maltophilia* in patients with AML in order to identify patients at increased risk for *S. maltophilia* infections.

METHODS

Patient Enrollment and Antibiotic Use Assessment

This was a *S. maltophilia*-focused substudy of a previously published microbiome-based prospective, observational, cohort study of patients with a new diagnosis of AML who were receiving remission-induction chemotherapy (RIC) between September 2013 and August 2015. Details on the cohort have been previously published [19, 20]. Seven patients from the original cohort were excluded from this analysis due to incomplete clinical and antimicrobial exposure data. Fecal and buccal microbiome samples were collected from each patient prior to the start of RIC and every 96 hours thereafter until the resolution of neutropenia (absolute neutrophil count >500 cells/mm^3). The University of Texas MD Anderson Cancer Center Institutional Review Board approved this study. All patients provided written, informed consent prior to enrollment in accordance with the Declaration of Helsinki.

Sample Collection and Microbiome Analysis

Buccal samples were collected using the Catch-All Sample Collection Swab (Epicentre) and placed in sterile 2-mL cryovials. Inpatient stool samples were collected in a stool hat and aliquoted into sterile 2-mL cryovials, while outpatient stool samples were collected using the BBL CultureSwab (BD Diagnostics). All samples were stored at −80° C until processing. Samples were submitted to the Alkek Center for Metagenomics and Microbiome Research (CMMR) of Baylor College of Medicine in 3 batches for microbial DNA extraction and microbiome profiling gene via 16S rRNA V4 gene sequencing. The CMMR is a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory that specializes in microbiome profiling and uses a set of controls to evaluate the performance of each step and determine potential contamination events throughout sample processing, library preparation, and data generation. Extraction controls are reagent controls (negative) and previously characterized samples (positive) that were subjected to the same procedures as the study samples. The 16S library preparation controls include a nontemplate control (negative) and purified DNA extracted from a pure culture of *Francisella tularensis* (positive). For the positive controls, 99% of reads are required to map to the *F. tularensis* reference strain in order to pass quality control. Both extraction and library preparation controls are carried through sequencing. For this study, data from extraction controls were not available due to the historical nature of the data. Additional information on control methods used for the microbiome analysis are presented in the Supplementary Methods. Bacterial DNA was extracted using the MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories), and 16Sv4 rRNA gene libraries were generated following a protocol adapted from the Earth Microbiome Project [21, 22]. Briefly, the 16S rRNA V4 gene region was amplified and sequenced using Illumina MiSeq using a 2 × 250 paired-end protocol. The 16S rRNA V4 gene sequences were assigned to operational taxonomic units (OTUs) using the UPARSE pipeline, and taxonomic classifications were derived from alignments to the SILVA SSURef_NR99_119 database.

Antibiotic Use Assessment, Definitions, and Statistical Analyses

All antibiotic use for each patient from the time of enrollment to completion of follow-up was extracted from a database maintained by the pharmacy informatics. An antimicrobial therapy day was defined as any single calendar day on which an antibiotic was administered, regardless of dose or dosing frequency. Antibiotic use was assessed at the individual drug level and considered as both any use (ie, 1 or more days of therapy) and cumulative use (ie, total days of therapy during the study period). Only antibiotics commonly used empirically to treat or prevent neutropenic fever were assessed to minimize selection bias. Prophylactic agents were ciprofloxacin, levofloxacin, and cefpodoxime; treatment antibiotics were cefepime, piperacillin-tazobactam, meropenem, linezolid, and vancomycin. As ceftazidime and tigecycline are rarely used during RIC and generally in patients at high risk for *S. maltophilia* infection at our institution, these agents were specifically not assessed. Patients were evaluated for infection and antibiotic use from start of chemotherapy until neutrophil recovery. Cultures were obtained following routine clinical practice. *Stenotrophomonas maltophilia* bacteremia was defined as growth of *S. maltophilia* from blood regardless of clinical symptoms or concomitant growth from any site other than blood. *Stenotrophomonas maltophilia* pneumonia was defined as growth of *S. maltophilia* from sputum or bronchoalveolar lavage (BAL) in the presence of new or changing pulmonary infiltrates and respiratory symptoms or a positive blood culture if no respiratory cultures were obtained. *Stenotrophomonas maltophilia* skin–soft tissue

infection (SSTI) was defined as skin erythema or swelling with growth of *S. maltophilia* from skin biopsy. Both *S. maltophilia* pneumonia and SSTI could exist independently of or concurrently with bacteremia.

The primary outcome was microbiologically documented infection with *S. maltophilia* (inclusive of bacteremia, pneumonia, or SSTI). Bivariate comparisons of patients with and without *S. maltophilia* infection were made using the Fisher exact test and the Mann-Whitney *U* test. A potential "best" predictive value of *S. maltophilia* relative abundance was determined by visually inspecting the receiver operator characteristics of each potential cut-point in order to maximize both sensitivity and specificity. In order to account for the time-varying nature of both *S. maltophilia* relative abundance and antibiotic use, a time-varying Cox proportional hazards model was used, with patients censored at neutrophil recovery or death. The time-varying Cox proportional hazards model accounts for immortal time bias and allows for an assessment of risk associated with each additional day of antibiotic exposure [23]. The last measured value was carried forward for patients with missing microbiome samples. A multivariable Cox proportional hazards model was constructed by starting with a full model and iteratively removing the least relevant predictors until an increase in the Akaike information criterion was observed. However, due to the limited sample size and likely overfitting, this model should be viewed as purely hypothesis-generating. All statistical analyses were performed using Stata v13.1 (StataCorp LP, College Station, TX).

RESULTS

Infection Characteristics

A total of 90 patients were included, 8 (8.9%) of whom developed microbiologically confirmed infection caused by *S. maltophilia*. Six patients had *S. maltophilia* pneumonia, 1 had ecthyma gangrenosum, and 1 had a complicated SSTI of the right lower extremity. One of 6 patients with pneumonia was diagnosed solely on the basis of a positive blood culture and development of nodular pulmonary infiltrates consistent with *S. maltophilia* infection. The remainder were diagnosed on the basis of bronchoalveolar lavage and/or respiratory cultures in addition to new or changing pulmonary infiltrates. Bacteremia was documented in 4 of 8 (50%) patients, including in 3 of 6 (50%) patients with pneumonia and in the patient with right lower extremity SSTI. Primary infection developed a median of 17.5 days (range, 11–28) following the start of induction chemotherapy. Clinical characteristics of patients with and without *S. maltophilia* infection are presented in Table 1, with no characteristics being significantly associated with *S. maltophilia* infection. The antimicrobial susceptibility profiles of the 8 diagnostic cultures are presented in Supplementary Table 1.

Stenotrophomonas maltophilia Microbiome Description and Relative Abundance

DNA extraction, 16Sv4 libraries, and 16Sv4 sequences were successfully generated for all the samples included in this analysis (438 stool and 556 oral). The 16S library polymerase chain reaction (PCR) nontemplate control yielded 75 sequencing reads; more than 75% mapped to *Methylobacterium*, a commonly identified laboratory and reagent contaminant [24], and none mapped to *Stenotrophomonas* or closely related genera (Supplementary Table 2).

Taxonomic classification and relative abundances of OTUs that mapped to the genus *Stenotrophomonas* were derived from the taxonomic classification table generated by the CMMR 16S pipeline. *Stenotrophomonas* spp. relative abundance was calculated as the percent of OTUs assigned to the genus *Stenotrophomonas* relative to all other assigned OTUs. Although there are at least 12 known species in the genus *Stenotrophomonas*, only 2 named species are included in the SILVA database *(Stenotrophomonas maltophilia* and *Stenotrophomonas pictorum)*. A BLASTn [25] analysis of the OTU sequences (2) mapping to the genus *Stenotrophomonas* in our data revealed 100% identity to *S. maltophilia* but also to *Stenotrophomonas pavanii*. Although v4 amplicons mapped to both *S. maltophilia* and another *Stenotrophomonas* species, *S. maltophilia* is the only member of this genera routinely identified in humans [26, 27].

Stenotrophomonas was detected in the oral or stool microbiome of only 3 (3.3%) and in none of the patients at baseline, respectively. *Stenotrophomonas* was detected at any point during the risk period (ie, the period between chemotherapy

Table 1. Baseline Characteristics of Patients With and Without *Stenotrophomonas maltophilia* Infection

Characteristic	No Infection (n = 82)	Infection (n = 8)	*P* Value
Age,[a] y	58 (46–68)	59 (56–72)	.27
Male sex	42 (51)	5 (63)	.72
High-intensity chemotherapy	55 (67)	5 (63)	1.00
Complex cytogenetics	10 (13)	3 (43)	.15
Eastern Cooperative Oncology Group (ECOG) performance status[a]	1 (1–1)	1 (1–2)	.20
Duration of neutropenia (days)[a]	26 (21–34)	29 (24–45)	.21

All reported as n (%) and tested with the Fisher exact test unless otherwise specified.

[a]Median (interquartile range); tested using the Wilcoxon rank sum test.

start and neutrophil recovery) in the oral and stool microbiome of 29 (32%) and 8 (9%) patients, respectively. Seven of 8 (88%) patients with *S. maltophilia* infection had oral microbiome detection of *Stenotrophomonas* prior to onset of infection in contrast to 22/82 (27%) without *S. maltophilia* infection ($P < .01$). The sole patient in whom *Stenotrophomonas* was not detected in the oral microbiome prior to infection had the last sample obtained 2 days prior to a diagnostic BAL; the oral sample obtained 2 days later had a relative abundance (ie, percentage of reads mapping to *Stenotrophomonas* relative to total number of reads) of 43%. The relative abundance of *Stenotrophomonas* in the oral microbiome varied over the duration of the risk period (Table 2) and tended to decrease after an initial peak (Figure 1). The median (interquartile range) maximum relative oral abundance of *Stenotrophomonas* was higher in patients with *S. maltophilia* infection (57% [1%–95%] compared with those with no infection (0% [0%–0%]; Figure 2). A peak oral *Stenotrophomonas* relative abundance of >36% appeared to best predict infection (sensitivity, 63%; specificity, 96%; likelihood ratio +, 17.08; likelihood ratio –, 0.39; positive predictive value, 61%; negative predictive value, 96%; 93% correctly classified). In contrast, any detection of *Stenotrophomonas* in the oral microbiome was a relatively poor predictor of *S. maltophilia* infection (sensitivity, 88%; specificity, 74%; likelihood ratio +, 3.26; likelihood ratio –, 0.17; positive predictive value, 24%; negative predictive value, 98%). Overall, 7/29 (24%) patients with any detection in the oral microbiome developed *S. maltophilia* infection. In contrast to the oral microbiome, there was no clear association between stool *Stenotrophomonas* detection and *S. maltophilia* infection, with 2/8 (25%) patients with infection having stool detection vs 6/82 (7%) without infection ($P = .15$). Further, the appearance of *Stenotrophomonas* in the fecal microbiome always followed its appearance in the oral microbiome (data not shown). When the time-varying relative abundance of *Stenotrophomonas* was considered, an increasing relative abundance of oral *Stenotrophomonas* colonization significantly correlated with *S. maltophilia* infection (Table 3).

Table 2. Oral and Fecal Microbiome *Stenotrophomonas* Relative Abundance in Patients With and Without *Stenotrophomonas maltophilia* Infection

Sample Site	No Infection (n = 82)	Infection (n = 8)	*P* Value
Oral			
Peak abundance (%)	0.00 (0.00–80.76)	57.27 (0.00–97.56)	<.01
Last abundance (%)	0.00 (0.00–54.56)	3.84 (0.00–96.57)	<.01
Baseline detection (n, %)	2 (2)	1 (13)	.25
Any detection (n, %)	22 (27)	7 (88)	<.01
Stool			
Peak abundance (%)	0.00 (0.00–9.85)	0.00 (0.00–92.14)	.07
Last abundance (%)	0.00 (0.00–9.85)	0.00 (0.00–0.63)	.24
Baseline detection (n, %)	0 (0)	0 (0)	1.00
Any detection (n, %)	6 (7)	2 (25)	.15

Values reported as median (range) unless otherwise reported. *P* values calculated using the Wilcoxon rank sum test (percent relative abundance) and Fisher exact test (percent detectable).

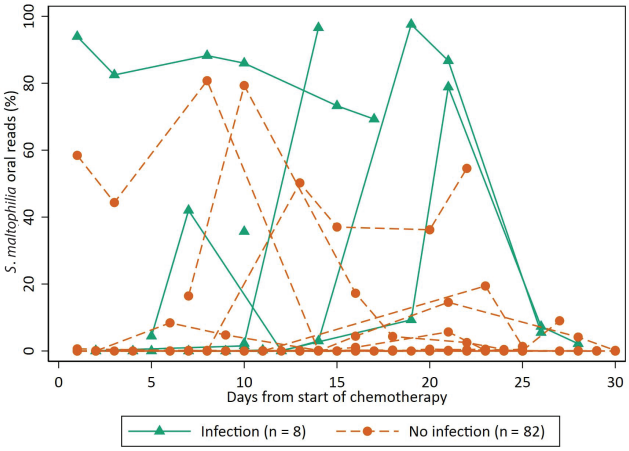

Figure 1. Relative abundance of *Stenotrophomonas* in patients with and without *Stenotrophomonas maltophilia* infection. All lines originate at the time of first sampling and end at the end of the risk period (time of *S. maltophilia* infection or neutrophil recovery). The *x*-axis is right-truncated at 30 days for clarity; 71/90 (88%) remained at 0% detectable throughout the risk period.

Antimicrobial Use Assessment

The use of antibiotics generally as initial treatment for or prophylaxis against neutropenic fever is presented in Table 4. When treated as a time-varying covariate, each additional day of meropenem use increased the hazard of *S. maltophilia* infection by 17% (hazard ratio [HR], 1.17; 95% confidence interval [CI], 1.01–1.35; $P = .03$). No other β-lactam antibiotic was significantly correlated with *S. maltophilia* infection (Table 3). Linezolid use also correlated with *S. maltophilia* infection (HR, 1.12; 95% CI, .99–1.27; $P = .06$), although this may be because linezolid receipt is highly correlated with meropenem. Indeed,

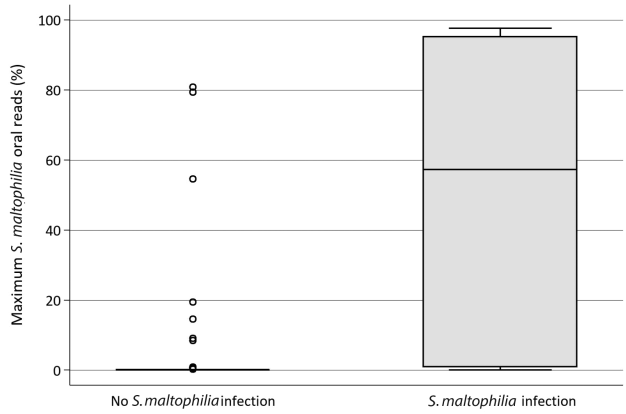

Figure 2. Maximum *Stenotrophomonas* oral abundance in patients with and without *Stenotrophomonas maltophilia* infection. Horizontal bars indicate median and upper and lower quartiles. Solid dots indicate outlier values, as applicable.

Table 3. Time-varying Antibiotic Exposure and Oral Microbiome Relative Abundance as Predictors of *Stenotrophomonas maltophilia* Infection

Antibiotic	Hazard Ratio	95% Confidence Interval	*P* Value
Cefepime[a]	1.02	.84–1.23	.87
Cefpodoxime[a]	1.00	.75–1.34	1.00
Ciprofloxacin[a]	0.75	.51–1.10	.15
Levofloxacin[a]	0.83	.66–1.04	.10
Linezolid[a]	1.12	.99–1.27	.06
Meropenem[a]	1.17	1.01–1.35	.03
Piperacillin-tazobactam[a]	1.07	.86–1.33	.55
Stenotrophomonas maltophilia oral abundance[b]	1.04	1.03–1.05	<.01

[a]Hazard ratios refer to hazard associated with each additional day of antibiotic exposure.

[b]Hazard ratios refer to hazard associated with 1% increase in *S. maltophilia* relative abundance.

in the exploratory multivariable model, meropenem and the relative oral abundance of *S. maltophilia* appear to be associated with increased risk of *S. maltophilia* infection, while ciprofloxacin and levofloxacin are associated with decreased risk (Table 5).

DISCUSSION

In this prospective, observational study, we identified the oral microbiome as a potential predictor of *S. maltophilia* infection in patients with AML who are receiving chemotherapy. Patients with *S. maltophilia* infection more frequently had detection of *Stenotrophomonas* in oral microbiome samples and had a higher relative abundance of *Stenotrophomonas* than patients without infection. Notably, this finding includes 2 patients with SSTIs caused by *S. maltophilia*, indicating that the oral microbiome may serve either as a potential reservoir for pathogenic *S. maltophilia* or as an indicator of multisite colonization pressure in these patients. Additionally, we confirm and expand on findings that carbapenems are a significant risk factor for *S. maltophilia* infection, identifying that each additional day of use further increases the risk of *S. maltophilia* infection [2, 9, 10].

Table 4. Antibiotic Use in Patients With and Without *Stenotrophomonas maltophilia* Infection

Antibiotic	No Infection (n = 82)	Infection (n = 8)
Cefepime		
Median (IQR) number of days	3 (0–7)	2 (0–5)
Any use (n, %)	49 (60)	4 (50)
Cefpodoxime		
Median (IQR) number of days	0 (0–2)	0 (0–0)
Any use (n, %)	25 (30)	1 (13)
Ciprofloxacin		
Median (IQR) number of days	0 (0–2)	0 (0–2)
Any use (n, %)	24 (30)	3 (30)
Levofloxacin		
Median (IQR) number of days	5 (0–10)	0 (0–2)
Any use (n, %)	55 (67)	3 (38)
Linezolid		
Median (IQR) number of days	6 (2–10)	8 (4–9)
Any use (n, %)	62 (76)	8 (100)
Meropenem		
Median (IQR) number of days	2 (0–8)	5 (3–10)
Any use (n, %)	43 (52)	7 (88)
Piperacillin-tazobactam		
Median (IQR) number of days	0 (0–2)	0 (0–3)
Any use (n, %)	23 (28)	2 (25)

Abbreviation: IQR, interquartile range.

Table 5. Exploratory Multivariable Analysis of Risk Factors for *Stenotrophomonas maltophilia* Infection

Factor	Adjusted Hazard Ratio	95% Confidence Interval	*P* Value
Ciprofloxacin[a]	0.59	.40–.87	<.01
Levofloxacin[a]	0.83	.62–1.12	.23
Meropenem[a]	1.10	.97–1.26	.12
Stenotrophomonas maltophilia oral abundance[b]	1.03	1.02–1.05	<.01

[a]Hazard ratios refer to hazard associated with each additional day of antibiotic exposure.

[b]Hazard ratios refer to hazard associated with 1% increase in *S. maltophilia* relative abundance.

Previous studies have clearly found that microbiome domination events precede infection with pathogenic microorganisms in patients with hematologic malignancy [16–18, 28, 29]. Importantly, however, these studies have focused on the fecal, rather than oral, microbiome. Our findings make it evident that the oral microbiome may play an important role in the pathogenesis of certain infections and should be given consideration in studies that link the microbiome with clinically relevant infections. It is worth noting previous studies that have identified associations between the fecal microbiome and subsequent infection have focused predominantly on infections caused by Enterobacteriales and *Enterococcus* spp., which in neutropenic patients are generally associated with primary bloodstream infection caused by gastrointestinal translocation [30]. In contrast, *S. maltophilia* infections in this population are generally either primarily respiratory in origin or catheter-related bloodstream infections; therefore, the relationship between the oral microbiome and *S. maltophilia* infection, rather than fecal microbiome and infection, does seem logical [3]. Whether the same relationship holds true for other organisms more commonly associated with respiratory infections rather than gastrointestinal translocation, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, is unclear.

We additionally expand on other studies that have assessed antibiotic exposure as a risk factor for *S. maltophilia* infection. Previous studies performed in general patient populations and in patients with hematologic malignancy have

identified carbapenem use as a significant risk factor for *S. maltophilia* infection, in agreement with the intrinsic resistance of *S. maltophilia* to carbapenem antibiotics [2]. However, not all studies have identified carbapenem use as a risk factor for *S. maltophilia* infection in all situations, particularly when evaluated as a dichotomized (ie, yes/no) variable [7, 10, 31]. In our study, we identified both dichotomized use of carbapenems as a risk factor for *S. maltophilia* infection as well as cumulative exposure assessed in a time-varying Cox proportional hazards model. It is reasonable to expect that a larger cumulative antibiotic exposure would increase risk to a greater extent than a smaller cumulative exposure. Indeed, the critical need to assess antibiotic exposure as a cumulative measure in a time-varying model has recently been demonstrated [23, 32, 33]. While it is possible and likely that prolonged length of stay is associated with more antibiotic use and exposure to hospital-acquired pathogens and could therefore potentially explain the observed association with cumulative antibiotic exposure, the differential cumulative risk of different antibiotics argues against this point. Of note, no other β-lactam antibiotic was associated with increased risk of *S. maltophilia* infection. As organisms that require the use of carbapenems are increasingly common in patients with AML, the risk–benefit trade-off for continued empiric carbapenem use relative to other β-lactams must be carefully considered in each patient [13].

The prevalence of *S. maltophilia* in patients with AML receiving induction chemotherapy is both surprising and concerning. While *S. maltophilia* is a known pathogen in cancer patients, it is generally perceived to be a pathogen that appears later in a patient's treatment course due to low virulence potential [10]. However, in this cohort of newly diagnosed patients with AML receiving induction chemotherapy, 9% of patients had microbiologically confirmed *S. maltophilia* infection and all infections occurred following receipt of a carbapenem. These data suggest that *S. maltophilia* infection must be considered in any patient with AML who has received treatment with a carbapenem, with patients who have received longer courses of carbapenems being at higher risk. Additionally, these data highlight the potential harms of early carbapenem use leading to microbiome dysbiosis and selection pressure for carbapenem-resistant organisms, such as *S. maltophilia*. As *S. maltophilia* is a fairly ubiquitous environmental organism and our data indicate that acquisition of *S. maltophilia* occurs over time, infection control measures or environmental screening may also be plausible methods to mitigate against the risk of early *S. maltophilia* infection [34].

There are several limitations to our study. First, the relatively small sample size and single-site nature of the study preclude the development of a multivariable risk prediction model, although preliminary findings indicate that such a model is feasible at scale. Due to the limited sample size, all results should be viewed as hypothesis-generating. Second, misclassification bias of our primary outcome, *S. maltophilia* infection, is possible as the diagnosis in many cases relied on respiratory cultures. In patients with heavy oral colonization by *Stenotrophomonas*, this may have led to contamination of the diagnostic respiratory culture. However, as half of the patients with pneumonia also had concomitant bacteremia and all patients had clinical signs and symptoms compatible with pneumonia, this seems less likely. It is unknown if these findings are applicable at other centers caring for patients with AML, and it is also not clear if the relationships between the microbiome, antibiotic exposure, and *S. maltophilia* infection are relevant in other patient populations. Additionally, the tremendous genetic heterogeneity of *S. maltophilia* is just beginning to be understood, and how interstrain variability may influence these findings is unknown [35]. Therefore, these findings require validation in a multicenter study. Finally, while the relationship between the oral microbiome relative abundance and *S. maltophilia* infection appears to be quite strong, the applicability of this finding is limited until longitudinal microbiome sampling on clinical samples becomes feasible as baseline detection does not appear to predict subsequent infection. However, for centers with a high prevalence of *S. maltophilia* infection, development of dynamic PCR-based screening methods may have utility in directing empiric treatment for patients with suspected infections, and the performance characteristics of such screening should be evaluated in future studies. Last, the lack of data on the extraction controls limited our ability to exclude the potential of a reagent or processing contamination event during DNA extraction or any processes upstream of it. In addition, techniques used for microbiome evaluation did not allow us to specifically determine that all sequencing reads that map to the genus *Stenotrophomonas* are, in fact, *S. maltophilia*. However, the absence of reads that map to *Stenotrophomonas* in the library preparation controls and the validation with species-specific PCR (data not shown) partially mitigate these concerns.

Despite these limitations, there are several notable strengths to our study. First, the prospective design and longitudinal microbiome sampling allowed for an assessment in the relative abundance of *Stenotrophomonas* as a function of time. Additionally, this cohort is the largest of its kind to date and can therefore provide insight on relatively rare individual events, such as *S. maltophilia* infection. Finally, incorporation of microbiome data and antibiotic use data represents a step forward in understanding how the interaction of the microbiome and antibiotic use may affect downstream infection risk.

In conclusion, the oral microbiome and cumulative antibiotic use appear to be important factors in the development of *S. maltophilia* infection in patients with AML receiving chemotherapy. Multicenter studies are needed to validate and expand on these findings.

Supplementary Data

Supplementary materials are available at *Clinical 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

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