

Microbiology of Bloodstream Infections in Children After Hematopoietic Stem Cell Transplantation: A Single-Center Experience Over Two Decades (1997–2017)

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Background. Bloodstream infections (BSIs) occur frequently after hematopoietic stem cell transplantation (HSCT). We examined the microbiology of BSI in pediatric HSCT recipients over a 2-decade period at our institution to inform empirical antimicrobial prescribing and infection prevention strategies.

Methods. We conducted a retrospective cohort study of children (<18 years) who underwent HSCT at Duke University between 1997 and 2015. We used recurrent-event gap-time Cox proportional hazards models to determine the hazards of all-cause and cause-specific BSI according to HSCT year. We compared the median time to BSI by causative organism type and evaluated for temporal trends in the prevalence of antibiotic resistance among causative organisms.

Results. A total of 865 BSI occurred in 1311 children, including 412 (48%) Gram-positive bacterial, 196 (23%) Gram-negative bacterial, 56 (6%) fungal, 23 (3%) mycobacterial, and 178 (21%) polymicrobial BSI. The hazard of all BSIs did not change substantially over time during the study period, but the hazard of fungal BSIs declined over time during the study period ($P = .04$). Most fungal BSIs (82%) occurred in the first 100 days after HSCT, whereas mycobacterial BSIs occurred later after HSCT than BSIs caused by other organisms ($P < .0001$). The prevalence of vancomycin resistance among BSIs caused by *Enterococcus faecium* increased during the study period ($P = .0007$). The risk of 2-year mortality in children was increased with BSI ($P = .02$), Gram-negative bacterial BSI ($P = .02$), and fungal BSI ($P < .0001$).

Conclusions. Despite expanded practices for BSI prevention over the past several decades, the incidence of BSI remains high in pediatric HSCT recipients at our institution. Additional strategies are urgently needed to effectively prevent BSIs in this high-risk population.

Keywords. antibiotic resistance; bloodstream infections; hematopoietic stem cell transplantation; immunocompromised host; pediatrics.

Bloodstream infections (BSIs) occur frequently after hematopoietic stem cell transplantation (HSCT) and are associated with substantial morbidity and mortality. Prior studies indicate that the cumulative incidence of BSI among HSCT recipients may approach 66% [1–5]. Up to two thirds of BSIs in these patients are associated with mucosal barrier injury and thus may not be effectively prevented by central venous catheter (CVC) insertion and maintenance bundles [6–8]. Recent studies conducted

in adult HSCT recipients suggest that the incidence of BSIs after HSCT declined over the past several decades [3, 9], although the proportion of infections caused by Gram-negative bacteria tended to increase [3, 10–12]. In addition, BSIs caused by antibiotic-resistant organisms are of particular concern in HSCT recipients because of frequent exposure to broad-spectrum antimicrobials. Recent studies reported that the incidences of BSIs caused by extended-spectrum beta-lactamase-producing bacteria, carbapenem-resistant Enterobacteriaceae (CRE), and vancomycin-resistant *Enterococcus* (VRE) are increasing in adult HSCT recipients [3, 9]. Studies describing the microbiology and antibiotic resistance of BSI after HSCT in children have been limited by small sample sizes and relatively short study periods, precluding evaluation of microbiological trends over time.

As the number of children undergoing HSCT increases, an improved understanding of the microbiology of BSI in this patient population is necessary to inform empirical antimicrobial

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prescribing and infection prevention practices. In this study, we describe the incidence and microbiology of BSIs among pediatric HSCT recipients at our institution over a 21-year period. As a secondary objective, we identify temporal trends in the prevalence of resistance to specific antibiotics among bacterial species that frequently caused BSIs in this cohort.

METHODS

Study Design and Population

We conducted a retrospective cohort study of children (<18 years of age) who underwent HSCT through the Duke University Pediatric Blood and Marrow Transplant Program between January 1, 1997 and December 31, 2015. The study protocol was approved by the Duke University Institutional Review Board.

Transplant Practices

Throughout the study period, patients did not receive routine antibacterial prophylaxis after HSCT. For *Pneumocystis jirovecii* prophylaxis, children received trimethoprim-sulfamethoxazole starting at the time of hospital admission and continuing until 2 days before HSCT, followed by inhaled or intravenous pentamidine starting 30 days after the HSCT date. Antifungal prophylaxis was administered routinely to patients throughout the study period. Before September 2003, low-dose amphotericin B lipid complex (0.2 mg/kg IV once daily) was routinely administered to allogeneic HSCT recipients. In September 2003, prophylaxis with voriconazole (4 mg/kg IV or PO twice daily) was implemented for most children undergoing allogeneic HSCT. Fluconazole was the most frequent antifungal agent used for prophylaxis among autologous HSCT recipients. Antifungal prophylaxis was started on the day after the HSCT date and continued for 100 days after HSCT or while the patient remained on immunosuppressive prophylaxis or therapy for graft-versus-host disease (GVHD). All patients had a double- or triple-lumen tunneled CVC placed before HSCT. Standard best practices for CVC care were routine and included daily bathing and antiseptic oral rinses while children were hospitalized and sterile care for CVCs, regardless of the patient's location. Throughout the study period, surveillance blood cultures were collected weekly (Sunday nights at midnight) from at least 1 CVC lumen from patients admitted to the hospital transplant unit. Finally, given the low incidence of BSI caused by anaerobic bacteria in children [13], anaerobic blood cultures were typically not collected from these patients.

Data Sources and Susceptibility Test Measures

We obtained data on patient demographics and transplant characteristics from a secure database maintained by the HSCT program. We identified positive blood cultures using the Duke Enterprise Data Unified Content Explorer (DEDUCE) research portal [14]. We reviewed patient electronic medical records

and an electronic database maintained by the Duke University Health System Clinical Microbiology Laboratory for antimicrobial susceptibility results for blood culture isolates. During the study period, antimicrobial susceptibility testing was performed for these bacterial species using Kirby-Bauer disk diffusion, broth dilution, Etest, or automated microdilution methods. We used 2019 Clinical and Laboratory Standards Institute (CLSI) breakpoints for zones of inhibition and minimum inhibitory concentrations (MICs) to classify isolates as susceptible/intermediate or resistant to a given antibiotic [15]. Given that this study spanned more than 2 decades, susceptibility testing for some antibiotics was not available for the entire study period. Most Gram-negative bacteria were routinely tested for susceptibility to piperacillin-tazobactam and meropenem starting in 2003. In addition, the laboratory transitioned from using oxacillin to using ceftiofuran for the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in 2004. Finally, early in the study period, some Enterobacteriaceae isolates were recorded as having a meropenem MIC of ≤ 4 $\mu\text{g/mL}$; we classified these isolates as susceptible/intermediate despite 2019 CLSI guidelines categorizing organisms with a meropenem MIC of 4 $\mu\text{g/mL}$ as resistant [15].

Definitions

We defined BSI in accordance with National Healthcare Safety Network (NHSN) criteria as follows: (1) growth of a recognized pathogen from blood culture, or (2) growth of a commensal organism (eg, coagulase-negative staphylococci, *Micrococcus* species) from 2 blood cultures drawn from different sites at the same time or from the same site at different times on the same or consecutive days [16]. To account for possible identification of contaminants in surveillance cultures, we excluded all commensal organisms identified in blood cultures collected between Sunday at 8:00 PM and Monday at 4:00 AM. Growth of the same or different organisms from blood cultures obtained within 14 days of the first positive blood culture in a BSI were considered to be from the same BSI episode. We identified all BSI episodes occurring in the study population during the 2 years after the HSCT date. If a patient received multiple transplants during the study period, the BSI was assigned to the transplant that most closely preceded the BSI episode. We classified BSI episodes as Gram-positive bacterial, Gram-negative bacterial, fungal, mycobacterial, or polymicrobial (containing more than 1 species).

Statistical Analyses

We used a Prentice-Williams-Peterson recurrent-event gap-time (PWP-GT) Cox proportional hazards model to compare the hazard of BSI by HSCT year category, adjusting for patient age, sex, HSCT indication, HSCT donor source, and conditioning regimen intensity [17, 18]. We categorized transplants into 4 periods based on HSCT year (1997–2000, 2001–2005,

2006–2010, and 2011–2015). The gap time was an interval that started at the time of the previous event (or HSCT for the first BSI episode) and ended at the time of the current BSI event or censoring event. Transplants were censored at 2 years after the date of HSCT, time of subsequent HSCT, or death. We similarly used PWP-GT Cox proportional hazards models to evaluate for an association between HSCT year category and the hazards of Gram-positive bacterial, Gram-negative bacterial, fungal, and polymicrobial BSI. Too few mycobacterial BSI occurred for similar analyses. Given the observed linear association between hazard ratios (HRs) for fungal BSI and year categories, we classified the year category variable as ordinal to evaluate for a change in the hazard of fungal BSI over time during the study period. An extended Cox model confirmed that the proportional hazards assumption was met for all BSI categories, with the exception of fungal BSI. Because there is no established method to account for nonproportional hazards in PWP-GT models, we present these model results with the known limitation of nonproportional hazards. We used a Kruskal-Wallis test to compare the time to BSI after HSCT by BSI category and Wilcoxon rank-sum tests for post hoc pairwise comparisons. We used Cochran-Armitage tests of trend to evaluate for a change in the proportion of antibiotic-resistant bacterial isolates over time during the study period. Finally, we calculated the relative risk (RR) of 2-year mortality associated with any BSI using a log-binomial regression model adjusted for patient age, sex, HSCT indication, HSCT donor source, conditioning regimen intensity, and HSCT year. We similarly determined the RR for 2-year mortality in patients with Gram-positive bacterial, Gram-negative bacterial, fungal, and polymicrobial BSI compared with those who did not experience the organism-specific BSI. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) and R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient Characteristics

Characteristics of the 1311 patients included in this study are shown in Table 1. Median age was 5.7 (interquartile range [IQR], 2.2–11.0) years, a majority (59%) of patients were male, and more than 70% of patients were of white race. The cohort underwent 1419 transplants during the study period. Most (83%) of these transplants were allogeneic, with umbilical cord blood representing the most frequent HSCT donor source. Hematological malignancies (42%), genetic or inherited metabolic disorders (20%), and solid tumors (16%) were the most frequent indications for HSCT.

Bloodstream Infection Episodes

We identified 865 BSI episodes occurring after 586 (41%) transplants during the study period. These BSI episodes were classified as Gram-positive bacterial (n = 412, 48%), Gram-negative

Table 1. Characteristics of the Study Population

Characteristics	n	%
Patients (n = 1311)		
Median (IQR) age, years	5.7	(2.2–11.0)
Sex		
Female	534	41%
Male	777	59%
Race		
Asian-Pacific Islander	51	4%
Black	202	15%
Hispanic	55	4%
Middle Eastern	41	3%
Other or not reported	22	2%
White	940	72%
Transplants (n = 1419)		
HSCT year		
1997–2000	327	23%
2001–2005	466	33%
2006–2010	347	24%
2011–2015	279	20%
HSCT Indication		
Genetic or inherited metabolic disorder	280	20%
Hematological malignancy	599	42%
Immunodeficiency	152	11%
Nonmalignant hematological disorder	162	11%
Other	4	<1%
Solid tumor	222	16%
HSCT Donor Source		
Allogeneic		
Bone marrow	314	27%
Umbilical cord blood	869	73%
Autologous	236	17%
Conditioning Regimen		
Myeloablative	1300	92%
Nonmyeloablative	68	5%
Reduced intensity	51	4%

Abbreviations: HSCT, hematopoietic stem cell transplantation; IQR, interquartile range.

bacterial (n = 196, 23%), fungal (n = 56, 6%), mycobacterial (n = 23, 3%), and polymicrobial (n = 178, 21%). Figure 1 shows the hazards of all BSI and specific BSI categories by HSCT year. Overall, the hazard of all BSI was relatively stable during the study period. Compared to 1997–2000, the hazard of all BSI was lower in 2001–2005 (HR = 0.83; 95% confidence interval [CI], 0.72–0.96), 2006–2010 (HR = 0.89; 95% CI, 0.77–1.02), and 2011–2015 (HR = 0.84; 95% CI, 0.71–0.998). No differences in the hazards of all BSI were observed between 2001–2005, 2006–2010, and 2011–2015. The hazards of Gram-positive bacterial BSI, Gram-negative bacterial BSI, and polymicrobial BSI were also largely stable over time. In contrast, the hazard of fungal BSI declined over time during the study period ($P = .04$).

We compared the timing of BSI episodes by causative organism category relative to the HSCT date (Figure 2). The median number of days after HSCT was 56 (IQR, 8–140.5) days for Gram-positive bacterial BSI, 67 (IQR, 10–161) days

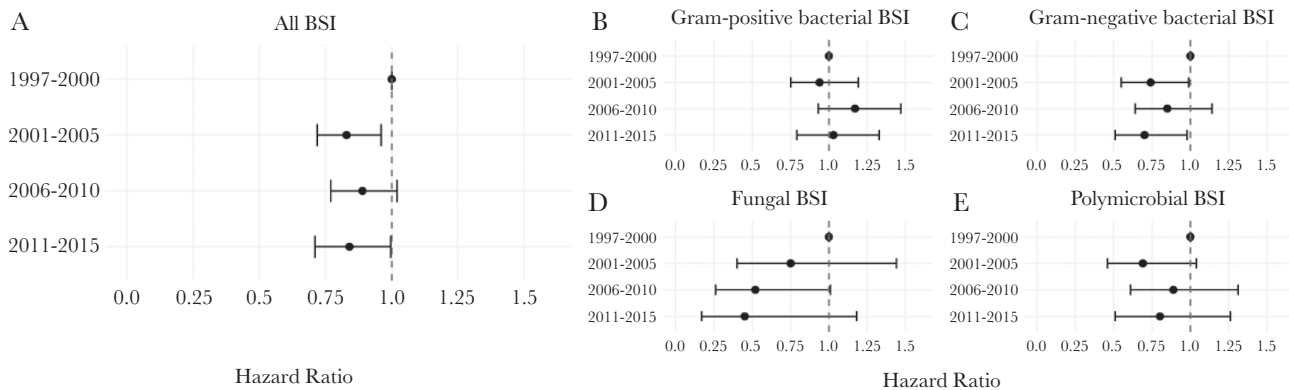


Figure 1. Forest plots for the risk of bloodstream infections (BSIs) among the study population by hematopoietic stem cell transplantation (HSCT) year. Forest plots of hazard ratios and 95% confidence interval bars with year category 1997–2000 serving as reference for the following: (A) all BSI, (B) Gram-positive bacterial BSI, (C) Gram-negative bacterial BSI, (D) fungal BSI, and (E) polymicrobial BSI.

for Gram-negative bacterial BSI, 42.5 (IQR, 25–68.5) days for fungal BSI, 151 (IQR, 107–511) days for mycobacterial BSI, and 74 (IQR, 24–138) days for polymicrobial BSI. The timing of BSI episodes after HSCT differed by BSI category ($P = .0006$). In particular, 46 of 56 (82%) fungal BSI episodes occurred during the first 100 days after HSCT, and mycobacterial BSI occurred later than other BSI episodes (median, 151 vs 62 days; $P < .0001$).

Bloodstream Infection Organisms

A total of 1114 organisms were isolated from the 865 BSI episodes (Table 2). Gram-positive bacteria accounted for more than half of the culture isolates, with coagulase-negative staphylococci ($n = 178$), *Enterococcus faecalis* ($n = 107$), and *Enterococcus faecium* ($n = 100$) being the most frequently identified species. Gram-negative bacteria accounted for one third of the culture isolates, with *Klebsiella pneumoniae* ($n = 53$),

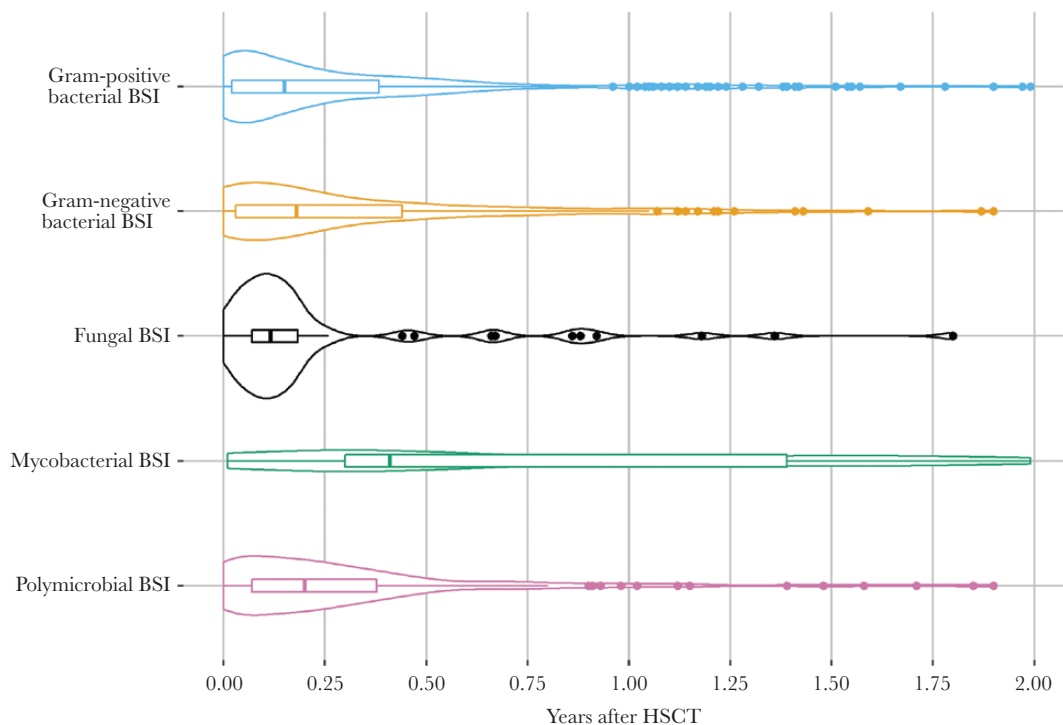


Figure 2. Timing of bloodstream infection (BSI) episodes relative to hematopoietic stem cell transplantation (HSCT). Violin plot depicting the time from HSCT to BSI by BSI category. Within the violin, we indicate the median and 25th and 75th percentiles. Bloodstream infection categories (Gram-positive bacterial, Gram-negative bacterial, fungal, mycobacterial, and polymicrobial) are mutually exclusive.

Table 2. Microbiology of Common Bloodstream Infection Causative Organisms (n = 1114)

Organism	n
Gram-Positive Bacteria	647
Coagulase-negative staphylococci	178
<i>Enterococcus faecalis</i>	107
<i>Enterococcus faecium</i>	100
Viridans group streptococcus	97
<i>Staphylococcus aureus</i>	72
Gram-Negative Bacteria	353
<i>Klebsiella pneumoniae</i>	53
<i>Enterobacter cloacae</i>	47
<i>Escherichia coli</i>	42
<i>Pseudomonas aeruginosa</i>	39
<i>Stenotrophomonas maltophilia</i>	26
Fungi	86
<i>Candida krusei</i>	19
<i>Candida albicans</i>	17
<i>Candida tropicalis</i>	14
Nontuberculous Mycobacteria	28
<i>Mycobacterium avium</i>	9
<i>Mycobacterium mucogenicum</i>	9
<i>Mycobacterium chelonae/immunogenum</i>	5

Enterobacter cloacae (n = 47), *Escherichia coli* (n = 42), and *Pseudomonas aeruginosa* (n = 39) being the most common Gram-negative bacterial isolates. All but 7 of the fungal culture isolates were *Candida*, with *Candida krusei* (n = 19) and *Candida albicans* (n = 17) being the most frequently identified species. Nontuberculous mycobacteria accounted for 28 (3%) of the blood culture isolates.

The prevalence of antibiotic resistance among several commonly isolated bacterial pathogens is shown in Table 3. The prevalence of vancomycin resistance among *E faecium* isolates increased during the study ($P = .0007$). In 1997–2000, 13% of *E faecium* isolates were vancomycin-resistant compared with 69% in 2011–2015. Seventy percent of *E faecium* isolates were resistant to ampicillin, although the prevalence of ampicillin resistance among *E faecium* did not change during the study period ($P = .88$). In contrast, all of the *E faecalis* culture isolates were susceptible to ampicillin with only 1 vancomycin-resistant *E faecalis* isolate identified. The prevalence of resistance to antistaphylococcal penicillins, such as methicillin, among *S aureus* was stable over time ($P = .35$), with 21% of isolates identified during the study period as MRSA. The prevalence of resistance to penicillin among viridans group streptococci declined during the study period ($P = .0005$). In 1997–2000, 88% of isolates were penicillin-resistant, whereas only 2 of 7 (29%) of the isolates identified in 2005–2010 and 2011–2015 were penicillin-resistant. The overall prevalence of resistance to piperacillin-tazobactam among the selected Enterobacteriaceae was low (11%) and did not change over time during the study period ($P = .45$). Only 1 meropenem-resistant Enterobacteriaceae

isolate was identified during the study period. The prevalence of gentamicin resistance among *Pseudomonas* spp declined over time during the study period ($P = .04$) from a peak of 29% in 1997–2000 to 0% among the 5 isolates identified in 2011–2015. The prevalence of resistance to ciprofloxacin, meropenem, and piperacillin-tazobactam among *Pseudomonas* spp was stable over time.

Mortality

Among the 1311 patients included in our cohort, all-cause 2-year mortality was 36%. Two-year mortality associated with BSI is shown in Table 4. Patients who had any BSI in the 2 years after HSCT had a higher risk of 2-year mortality than patients who did not have a BSI (RR = 1.19; 95% CI, 1.03–1.37). In comparing the risk of 2-year mortality among the different BSI categories, we found an increased risk of mortality among patients who had a Gram-negative bacterial BSI (RR = 1.24; 95% CI, 1.04–1.48) or a fungal BSI (RR = 1.82; 95% CI, 1.54–2.17) compared with patients who did not have Gram-negative bacterial or fungal BSI, respectively. There was no increase in mortality risk associated with Gram-positive bacterial BSI or polymicrobial BSI.

DISCUSSION

We describe the microbiology of 856 BSI episodes and antibiotic resistance among 1114 causative organisms in the largest cohort of pediatric HSCT recipients reported to date. We observed a high incidence of BSIs in this patient population and a relatively stable risk of BSI over time during a study period spanning more than 2 decades. Mycobacterial BSIs occurred later after HSCT than other BSIs, whereas the vast majority of fungal BSIs occurred during the first 100 days after HSCT. We observed a notable shift in the prevalence of vancomycin resistance among *E faecium*. Finally, we found an increased RR of 2-year mortality in children who experienced any BSI, and specifically with Gram-negative bacterial and fungal BSIs.

We found that the 2-year cumulative incidence of BSIs among children and adolescents who underwent HSCT at our institution was 41%. This incidence is comparable to other pediatric and adult cohorts, with cumulative incidences in pediatric cohorts ranging broadly from 15% to 65% [1, 4, 19–22]. Previous studies reported an increase in BSI in association with specific risk factors, including allogeneic HSCT, unrelated HSCT donor, myeloablative conditioning, total body irradiation, and delayed engraftment [1, 20, 23–25]. Although we did not directly assess the contribution of these factors to the risk of BSI, our cohort is a unique population that has a high prevalence of several of these risk factors. It is notable that the majority of pediatric transplants performed at our institution are allogeneic, and approximately three quarters of these transplants are from an unrelated umbilical cord blood donor, which has been associated

Table 3. Prevalence of Antibiotic Resistance Among Common Bacterial Pathogens Over Time During the Study Period

Organism and Antibiotic	HSCT Year Category						P ^a	
	1997–2000		2001–2005		2006–2010			2011–2015
	Total	%Resistant	Total	%Resistant	Total	%Resistant	Total	%Resistant
Gram-Positive Bacteria								
<i>Enterococcus faecalis</i>								
Ampicillin	24	0%	28	0%	36	0%	18	0%
Vancomycin	24	0%	28	0%	36	0%	18	6%
<i>Enterococcus faecium</i>								
Ampicillin	16	63%	22	77%	34	71%	26	69%
Vancomycin	16	13%	22	45%	34	53%	26	69%
<i>Staphylococcus aureus</i>								
Nafcillin	16	13%	26	15%	19	32%	11	27%
Viridans Group Streptococci								
Penicillin	8	88%	15	80%	6	33%	1	0%
Gram-Negative Bacteria								
Enterobacteriaceae								
Meropenem	ND	ND	26	0%	53	2%	29	0%
Piperacillin-tazobactam	ND	ND	26	19%	53	8%	29	10%
<i>Pseudomonas</i> spp								
Ciprofloxacin	14	14%	22	18%	18	11%	5	0%
Gentamicin	14	29%	22	9%	18	6%	5	0%
Meropenem	1	100%	18	11%	18	11%	5	0%
Piperacillin-tazobactam	ND	ND	16	6%	18	6%	5	0%

Abbreviations: HSCT, hematopoietic stem cell transplantation; ND, not done.

^aCochran-Armitage test of trend P value.

Table 4. Two-Year Mortality Associated With All BSI and Organism-Specific BSI (n = 1311)

Type of BSI	n	Deaths	Mortality	RR	P ^a
All BSI					
No BSI	750	237	32%	1.19 (1.03–1.37)	.019
Any BSI	561	230	41%		
Gram-Positive Bacterial BSI					
No BSI	982	336	34%	1.07 (0.91–1.25)	.425
Any BSI	329	131	40%		
Gram-Negative Bacterial BSI					
No BSI	1143	391	34%	1.24 (1.04–1.48)	.018
Any BSI	168	76	45%		
Fungal BSI					
No BSI	1263	431	34%	1.82 (1.54–2.17)	<.0001
Any BSI	48	36	75%		
Polymicrobial BSI					
No BSI	1162	407	35%	1.09 (0.87–1.34)	.416
Any BSI	149	60	40%		

Abbreviations: bloodstream infection; RR, relative risk.

^aAdjusted log-binomial model *P* value.

with a higher risk of infection in several prior cohorts [19, 26]. In addition, more than 90% of children undergoing HSCT at our institution receive myeloablative conditioning, which has been associated with a higher incidence of BSIs compared with nonmyeloablative conditioning [21, 25]. Given the large variability of BSI incidence by institution, transplant practices, and HSCT characteristics, larger multicenter studies are required to accurately quantify the risk of BSI in pediatric HSCT recipients.

Contrary to our results, prior studies showed a decrease in all BSI over time in HSCT recipients, although these studies primarily evaluated adults who received antibiotic prophylaxis after HSCT [3, 9, 27]. It is interesting to note that another study of pediatric HSCT that spanned 18 years and used similar statistical methods did not find a significant change in the risk of all BSI during the time that HSCT recipients were on immunosuppressive therapy [1]. Unlike our findings in this report, a recent retrospective study conducted by our team revealed a decline in the cumulative incidence of hospital-associated BSI occurring during the transplant hospitalization of pediatric HSCT recipients over the course of a 20-year study period [5]. In that study, the median length of hospitalization was 36 (IQR, 27–52) days and as such, it had a more limited window of time at risk for BSI than in the current study. Consistent with later, posthospitalization, community-acquired origins for many of the BSI in this study, a review of BSI over a 25-year time period in adults revealed that 39% of BSI after HSCT were nonnosocomial in origin, and in a pediatric cohort of HSCT recipients, 59% of central line-associated bloodstream infections were community-acquired [4, 27]. Taken together, this likely indicates that there is a continued, substantial risk of BSI extending well after the transplant hospitalization that could account for the difference in results between the 2 retrospective studies from our institution [5]. Bloodstream

infection prevention strategies should be optimized in the outpatient setting to further improve infectious outcomes in these high-risk children.

With regard to causative organisms, Gram-positive bacteria accounted for approximately half (48%) of the BSI episodes identified in our cohort, a finding that is consistent with prior studies conducted in HSCT recipients [3, 9, 27–29]. Coagulase-negative staphylococci are a common cause of BSI after HSCT [9, 11, 24] and were the most frequently isolated organisms in our study, likely related to the nearly universal use of CVC in this population. Viridans group streptococci have also historically been among the most common causes of BSI in this population but accounted for only 9% of BSI organisms in our cohort [10, 19, 30, 31]. Several recent studies in HSCT recipients reported that the incidence of BSI caused by viridans group streptococci has decreased over time, perhaps as an effect of improved oral care and use of reduced-intensity conditioning regimens [10, 32]. A number of studies of HSCT recipients reported that the proportion of BSI episodes caused by Gram-negative bacteria has increased over the past several decades [3, 10–12, 23, 27, 29, 33]. In our cohort, the incidence of Gram-positive bacterial BSI was approximately twice that of Gram-negative bacterial BSI, comparable to these previous studies. However, the risk of Gram-negative bacterial BSI was stable during the 2-decade study period. This is fortunate, given the increased risk of 2-year mortality in our cohort and the high morbidity and mortality that has previously been associated with Gram-negative bacterial BSI [11, 34].

Fungal and mycobacterial BSI are comparatively infrequent in HSCT recipients but are associated with substantial morbidity and mortality. Few prior studies described the epidemiology of fungal BSI after HSCT; however, existing epidemiology

suggests a risk of these infections to be up to 1% of HSCT recipients [35, 36]. In our cohort, fungi constituted a substantial etiology for BSI at 6%. *Candida* species were the most common fungal organisms isolated. The vast majority of fungal BSI in our cohort occurred before day 100 after HSCT, which corresponds to the period during which severe neutropenia, mucosal barrier injury, and acute GVHD are most common [37, 38]. We also found a decline in the hazard of fungal BSI over the 21-year study period. This may be due to the transition in institutional antifungal prophylaxis from low-dose liposomal amphotericin B to voriconazole, because a lower incidence of invasive fungal infections with voriconazole compared with liposomal amphotericin B has been described in pediatric HSCT recipients [39]. Nontuberculous mycobacterial infections occur in 3%–6% of allogeneic HSCT recipients and are often associated with chronic GVHD [40, 41]. Consistent with this observation, we found that mycobacterial BSI occurred at a median of 151 days after HSCT, a period that is classically associated with timing of chronic GVHD [42].

Antibiotic resistance among BSI pathogens remains a clinical challenge, particularly among medically experienced patients like those undergoing HSCT. In our cohort, the prevalence of vancomycin resistance among *E faecium* rose substantially during the study period. Vancomycin-resistant *Enterococcus* BSI is a known critical concern among adult HSCT recipients. It has become the most common BSI causative organism in allogeneic HSCT recipients at some institutions, with a VRE BSI 7-day mortality similar to that of Gram-negative bacterial BSI [43, 44]. Despite limited literature on VRE infections among children, VRE is now a commonly encountered infection among hospitalized children, especially in intensive care units and pediatric oncology wards [45, 46]. To our knowledge, this study is the first to demonstrate a rise in vancomycin resistance among *E faecium* over time in a pediatric HSCT cohort. This is particularly concerning because VRE colonization and infection are associated with risk factors that are almost unavoidable in patients undergoing HSCT, particularly immune suppression, broad-spectrum antibiotic exposure, and prolonged hospitalizations [45]. In the adult HSCT population, VRE BSI is associated with a worse overall survival; however, mortality is infrequently attributable to the VRE BSI [47]. Indeed, multiple investigators have shown no benefit in patient outcomes with linezolid as empirical therapy for fever and neutropenia [48, 49]. Thus, VRE prevention strategies should be prioritized over simply providing broader empirical therapy.

Antibiotic resistance was otherwise stable over the study period. We did not see an increase in the prevalence of methicillin resistance among *S aureus* during the study, which mirrors the Centers for Disease Control and Prevention's most recent report of a continued decrease in the number of infections due to MRSA in recent years [50]. Finally, unlike Bock et al [24], there was stable antibiotic resistance among the Gram-negative

isolates. We found a low, stable resistance to piperacillin-tazobactam and almost no CRE in our cohort. This is perhaps due in part to our institutional practice of using cefepime for empirical treatment of fever and neutropenia and the association of routine cefepime use with decreasing resistance to piperacillin-tazobactam that has previously been observed in hospitalized children [51].

Consistent with previous reports, we found an increase in all-cause mortality at 2 years among patients who experienced at least 1 BSI compared with patients without BSI [10, 52]. This was likely due to the high mortality rate in children who had Gram-negative bacterial and fungal BSI in our cohort. Kern et al [52] previously reported that Gram-negative bacterial BSI, fungal BSI, and enterococcal BSI were associated with higher 30-day mortality in adults with neutropenia after induction chemotherapy for acute leukemia or HSCT. Despite the frequency of *Enterococcus* spp as a cause of BSI in our cohort, we did not find an increase in mortality associated with Gram-positive bacterial BSI. This is perhaps due to the low virulence of other frequent causes of Gram-positive bacterial BSI, particularly coagulase-negative staphylococci. Although we did not directly assess BSI as a cause of mortality, Blennow et al [10] found a low attributable mortality of BSI and a relatively high crude mortality associated with BSI. This suggests that, although BSI may not be the direct cause of death in children after HSCT, it may be associated with other risk factors for mortality, including GVHD, in this patient population [53].

Our study has a few notable limitations. First, it was a single-center study and may not be generalizable to other institutions with different clinical practices and regional microbiological and antibiotic resistance patterns. The HSCT recipients at our institution do not routinely receive antibiotic prophylaxis and frequently receive broad-spectrum empirical antibiotics, which may impact the incidence of BSI and prevalence of antibiotic resistance. Many of the HSCT performed at our institution are from unrelated umbilical cord blood donors, which would likely affect the generalizability of our results from this unique cohort. We did not specifically identify mucosal barrier injury-laboratory confirmed bloodstream infections nor did we evaluate the effect of specific CVC practices on BSI incidence. In addition, despite this being the largest pediatric HSCT cohort published to date, our power to detect temporal changes in the risk of specific causes of BSI and the prevalence of antibiotic resistance was limited. Finally, we were unable to determine the susceptibility of some bacteria to specific antibiotics because of how antimicrobial susceptibility data were reported by our clinical microbiology laboratory. In particular, because of a change in the reporting of MICs for ceftriaxone during the study period, we were unable to evaluate for a temporal change in ceftriaxone resistance among Enterobacteriaceae. In addition, a large proportion of viridans group streptococci isolates

were not tested for antibiotic susceptibility, despite meeting current NHSN BSI criteria.

CONCLUSIONS

In conclusion, we describe the microbiology and antibiotic resistance of BSI episodes in a large cohort of pediatric HSCT recipients spanning 2 decades of transplantation. Given that we found a sizable incidence of BSIs among our cohort with no significant decline in risk over a 2-decade study period, our results suggest that future infection prevention strategies should more effectively target BSI prevention to reduce a significant source of infection in these high-risk patients. This will likely include indwelling CVC care, improved skin and oral care, and acute and chronic GVHD prophylaxis and treatment to be continued even after hospital discharge. There are currently insufficient data to support routine antibacterial prophylaxis for children undergoing HSCT, but this remains an active area of research [54]. Finally, the rising prevalence of VRE in this cohort is particularly worrisome. Our institution and others should consider the rising prevalence of VRE as an emerging infectious threat in pediatric HSCT recipients and make VRE infection prevention strategies a priority.

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Author contributions. S. M. H., I. C. A., R. R. Y., H. H., and M. S. K. designed the study and performed data collection, data interpretation, and initial manuscript review. J. S. T., L. E. M., K. J., and S. M. H. collected and interpreted data. R. R. Y. and S. M. H. performed data analysis. P. L. M. and P. C. S. interpreted data. S. M. H. prepared the initial draft of the manuscript. All authors critically reviewed and contributed to subsequent versions before approving the final manuscript for submission.

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