Evolution, Incidence, and Susceptibility of Bacterial Bloodstream Isolates from 519 Bone Marrow Transplant Patients

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Bacteria remain an important cause of infection in bone marrow transplants. To examine shifts in the etiology and susceptibility of bacterial isolates from transplants, we reviewed the incidence and susceptibility of blood isolates during a 7-year period. The infection rate fell dramatically during this time. Gram-positive organisms were isolated more often than gram-negative organisms, but the trend is reversing. Streptococci surpassed staphylococci for 5 years as the leading pathogen. Increasing resistance to penicillin, ciprofloxacin, and imipenem was noted in *Streptococcus* species. With the exception of type 1 β-lactamase–producing bacteria and *Pseudomonas aeruginosa*, gram-negative isolates remained overall susceptible to ceftazidime. Increased antibiotic prophylaxis coincided with the reduction in percentage of infected patients and increase in resistance to β-lactam antibiotics. Mortality attributed to bacteremia was low except for infections caused by *P. aeruginosa* and the *Enterobacter, Serratia, Citrobacter* group. There was no mortality attributable to gram-positive organisms such as *Staphylococcus aureus* and viridans streptococci.

Infection remains an important cause of morbidity and mortality in febrile neutropenia [1]. Bacteremia is the estimated cause of fever in 25% of all neutropenic patients [2] and fungemia or localized fungal infections, which tend to occur later for ~5% of fevers [3]. The bone marrow transplant (BMT) patient is infected with the same initial organisms as the general neutropenic patient, but organisms such as viridans streptococci are more frequent causes of bacteremia. In addition, neutropenia in BMT recipients is associated with a higher mortality than in nontransplant neutropenic patients [3–5]. This may be explained by the more profound and more prolonged duration of neutropenia, which may last >3 weeks in allogeneic recipients [6]. Other factors that influence mortality in the BMT patient include the occurrence of graft versus host disease and cytomegalovirus infections, but they do not occur during the neutropenic period immediately following transplantation.

In the 1970s, gram-negative organisms accounted for 70% of documented bloodstream pathogens in febrile neutropenia, and mortality due to gram-negative sepsis was reported to be as high as 40% [7]. The introduction of prophylactic antimicrobial agents against gram-negative pathogens, more intensive chemotherapy causing severe mucositis, and the increased use of long-term indwelling intravascular devices probably contributed to a change in the organisms isolated from bacteremias. By the mid 1980s, gram-positive organisms accounted for ~70% of all infections and gram-negative organisms for 30% [2, 8]. Coagulase-negative staphylococci (CoNS) replaced *Staphylococcus aureus* as the most common gram-positive pathogen, and *Escherichia coli* and *Klebsiella* species remained the most commonly isolated gram-negative organisms. *Pseudomonas aeru-
ginosa bacteremia decreased, but unusual and more resistant pathogens, such as Acinetobacter species and Stenotrophomonas maltophilia, were increasingly isolated [9]. Whereas most of these observations were made in general populations of febrile neutropenic patients, few studies have focused on BMT recipients [9–11]. The purpose of this study was therefore to describe the epidemiological and susceptibility changes of bacterial blood isolates over a 7-year period (1991–1997) in the bone marrow transplant unit (BMTU) at the University of Florida.

METHODS

Patients. Shands Hospital is a 576-bed teaching hospital of the University of Florida, Gainesville. The BMTU grew in size during this period from an 8-bed to a 20-bed unit. The transplanted population was between <1 year to 67 years of age. Pediatric patients (<18 years of age) accounted for 28% of the patients during the study period. The transplant population from which these data were collected consisted of ~35% leukemias, 25% lymphomas and multiple myelomas, 30% nonhematological malignancies, and 10% other pathologic conditions involving the bone marrow or immunodeficiency syndromes. Autologous and allogeneic transplants accounted for 60% and 40% of all procedures, respectively. Between January 1991 and December 1997, 519 BMTs were performed. Patients who underwent >1 BMT procedure were counted as different patients. The number of yearly transplants gradually increased from 50 for the 1991 calendar year to 150 in 1997.

Antibiotic use policy. There was no specific antibiotic prophylaxis policy during the period under review, nor was prophylaxis limited to a single agent. Prophylaxis was used increasingly beginning in 1994 and generally consisted of the administration of either a quinolone- (mostly ciprofloxacin), trimethoprim-sulfamethoxazole-(TMP-SMX), or β-lactam–type drug (penicillin or cefazolin). Macrolides were also used increasingly in some patients with occasional rifampin use. When a neutropenic patient (absolute neutrophil count <500 neutrophils/mm³ or trending down) became febrile, antibiotics were switched from prophylactic regimens to empiric therapeutic regimens. Antibiotic coverage generally consisted of monotherapy with ceftazidime or imipenem or rarely combination therapy with one of those agents plus an aminoglycoside or vancomycin. Vancomycin was used extensively in patients for persistent fevers, or when a gram-positive pathogen was isolated from the blood, pending sensitivity testing and when a catheter-associated infection was suspected.

Blood cultures and bacteremia. Blood cultures were obtained when the patient developed fever and often daily thereafter for the duration of the febrile episode. A blood culture was considered to be positive if ≥1 bottle grew an organism, with the exception of CoNS, which required 2 separate positive blood cultures to be considered a true bacteremia. A primary bacteremia was defined as a positive blood culture obtained while a patient was receiving prophylactic antibiotics or no antibiotics, or within 48 h of beginning antibiotics. A superinfection was defined as a positive blood culture occurring after the patient has received a minimum of 2 days of empiric therapy for fever and with a different organism if there was an initial isolate. The site of collection (peripheral or from a catheter site) was not analyzed in this study.

Bacterial data collection. All positive blood cultures from patients undergoing a BMT during the period 1991–1997 were reviewed. The date and type of each bloodstream isolate was obtained from the infection control computerized records. As policy, the infection control staff monitors each patient with a positive blood culture in the BMTU then determines whether this is clinically significant. Criteria for clinical significance included that the patient be febrile or show evidence of sepsis (with increased heart rate and hypotension) or that there be evidence of a line infection. It can be assumed that each recorded bloodstream infection has a high probability of representing a true bacteremia. The list of positive blood cultures was cross-matched for accuracy with the microbiology computer records. Sensitivity data were obtained from the computerized microbiology database, patient charts, and the original microbiology records. Antibiotic sensitivities were determined by the Kirby-Bauer disk diffusion method or by an automated microdilution method, using the National Committee for Clinical Laboratory Standards criteria for susceptibility. Streptococci were tested by the Kirby-Bauer method, using Mueller-Hinton blood agar plates. Ceftazidime was not routinely tested against streptococcal species because this agent was not a drug to be used in such cases. Penicillin testing, however, was routine. Organisms reported as fully susceptible to an antimicrobial agent were considered to be sensitive. Organisms reported as intermediate or resistant were considered resistant. Subsequent isolations of the same pathogen were considered to be the same episode of bacteremia and therefore not counted again. If a different pathogen was isolated, however, or if a blood culture was polymicrobial, each isolate was counted separately in order to obtain the frequency of a given isolate in this population. Antibiotic usage for each patient was obtained from the in-hospital pharmacy daily billing sheets and from patient charts.

RESULTS

Patient Demographics

From January 1991 to December 1997, 519 BMTs were performed. A total of 250 bacteria were isolated from the blood of 189 patients. A total of 138 patients had a bloodstream infection with a single pathogen, 39 patients had 2 pathogens,
and 12 patients had 3 or more pathogens isolated from their bloodstream. Of the 189 patients with bacteremias, 45% were recipients of an autologous transplant and 55% were allogeneic transplants. Approximately 32% of patients were between 1 and 20 years of age, with equal percentages for the age ranges between 21 and 40 years and 41 and 60 years. The overall male to female ratio was 1.0.

**Trends and Incidence of Bacterial Isolates**

While in the hospital, 29.5% of patients developed a primary bacterial infection, whereas bacterial superinfections occurred in 7.5% of patients. The average time from the day of the transplant procedure to the first positive blood culture was 7.5 days. Bacterial infection occurred within 5 days of bone marrow infusion in ~35% of the patients, 75% within 10 days, and 86% within 21 days. Of the 250 isolates, 155 (62%) were gram-positive bacteria and 38% were gram negative. On a yearly basis, the gram-positive organisms have outweighed the gram-negative organisms, although the ratio of gram-positive organisms to gram-negative organisms has declined from 2.7 to 1.3 (figure 1). In the 1991 period, gram-positive organisms accounted for 69.2% of all bacterial infections, whereas in the 1996 and 1997 period the percentage had fallen to 56%.

**Bacterial Isolates (1991–1997)**

**Gram-positive isolates.** Among the gram-positive isolates (primary and superinfections), *Streptococcus* species, primarily *S. mitis* and *S. sanguis* accounted for 49% of gram-positive bacteria, followed by CoNS and *S. aureus* (42.5%). *Enterococcus* species accounted for 7% of isolates. *Listeria monocytogenes*, *Bacillus* species, and *Corynebacterium* species accounted for 1.5% of the isolates. There were only 3 *S. pneumoniae* isolates. *Streptococcus* species was the most common isolated gram-positive organism in 5 of the 7 years, comprising 34%–75% of isolates (table 1). The incidence of *Streptococcus* species blood isolates in all patients was 14.6%. This compares to 12.3% for *Staphylococcus* species (9.2% for CoNS). On a per-year basis, the percentage of BMT recipients diagnosed with a primary bacteremia due to gram-positive pathogens has dramatically declined (figure 2). This decline was most dramatic with the *Streptococcus* species and the *Staphylococcus* species, which saw declines from the 1991–1992 period from 22%–32% and 21%–24%, respectively, down to 5% and 3% of patients being infected in the 1997 calendar year (figure 2). This decline was in parallel with the overall decrease in the isolation of bacteria from the BMT population.

**Gram-negative isolates.** Among all gram-negative bacteremias, *E. coli* and *Klebsiella* species were the most commonly isolated bacteria (*n* = 49) and together accounted for 51.5% of the gram-negative organisms. *Enterobacter* species, *Citrobacter* species, and *Serratia marcescens* organisms that produce the Bush group 1 β-lactamase made up 17.8% (*n* = 17), and *P. aeruginosa* 14.7% (*n* = 14), of the isolates, respectively. Other infrequently isolated bacteria made up the remaining 16% (table 1). The incidence of infection by each group of organisms for all patients was 9.4% for *E. coli* and *Klebsiella* species, 3.2%, for the *Enterobacter, Citrobacter, Serratia* group, and 2.7% for *P. aeruginosa*. Again, similar results are obtained when only

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**Table 1. Number of strains isolated from bone marrow transplant patients during the 1991–1997 period, by year.**

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<td>52</td>
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Figure 1. Percentage of gram-positive organisms versus gram-negative organisms isolated from bacteremias over the 7-year period.
primary infections are considered. *E. coli* and *Klebsiella* species infected 8.7% of patients, *Enterobacter*, *Serratia* and *Citrobacter* 2.1%, and *P. aeruginosa* 1.5%. These latter numbers therefore show the risk on specific types of gram-negative bacteria causing initial fevers in our population during this period. The yearly incidence of *E. coli* and *Klebsiella* species has remained between 6% and 11%. The incidence of the *Enterobacter*, *Citrobacter*, *Serratia* group ranged from 1% to 7%. The incidence of *P. aeruginosa* isolates in the overall BMT population remained <2% for the 6-year period from 1992 to 1997.

Uncommon isolates. Inherently multiresistant opportunistic pathogens have been described in hematology patients [9]. In our unit multiresistant isolates consisted of *Acinetobacter* species (2 isolates), *S. maltophilia* (1), *Alcaligenes xylosoxidans* (1), *Bacillus* species (1), and *Corynebacterium* species (1). Other relatively sensitive uncommon pathogens isolated included *Capnocytophaga* (3), *Listeria monocytogenes* (2), *Bacteroides fragilis* (4), and *Hemophilus influenzae* (1). These uncommon organisms accounted for ~5% of all isolates.

Polymicrobial bacteremia. Of the 51 patients who had >1 pathogen isolated during their hospital stay, 36 had 2 organisms isolated from a single blood culture. *E. coli*, *B. fragilis*, and *Enterococcus* species were isolated with another organism ~50% of the time. Approximately half of the polymicrobial *E. coli* isolates were associated with a *Streptococcus* species (8 of 15 isolates).

Mortality
Mortality was considered attributable to the bloodstream pathogen if the patient died within 7 days of bacteremia and there was no other ascertainable reason. Among aerobic gram-negative pathogens, *P. aeruginosa* had the highest associated mortality rate (40%) (table 2) followed by the *Enterobacter*, *Citrobacter*, *Serratia* group with 25% mortality. Only 1 of 33 patients with *E. coli* or *Klebsiella* species died within a 7-day period. Death from gram-positive isolates was almost nonexistent. Not a single patient with *S. aureus*, enterococci, or *Streptococcus* species isolates died. Three of 39 patients with CoNS bloodstream isolates (8%) died within 7 days of bacteremia. Two of 36 patients (6%) with polymicrobial bacteremia died within 7 days.

Susceptibility
The susceptibilities of 91.5% strains was available for review. The data were pooled for periods so that the trends could be ascertained when large numbers of isolates were available. For smaller numbers of isolates, the overall resistance during the period was calculated.

Gram-positive organisms. The resistance of the *Streptococcus* species to commonly used antibiotics were grouped in 3 time periods (figure 3); the number of organisms tested is shown in the figure. Thirteen percent (10/76) of the *Streptococcus* species were nonviable on subculture out of the blood culture bottles, and no sensitivities were reported. The data reflect a significant increase in penicillin resistance, from 7% in the period from 1991 to 1993, to 19% in the 1994–1995 period, to 58% in the 1996–1997 period. Imipenem resistance was noted in 25% of the reported isolates in the 1996–97 period, with no resistance noted in the prior years. Resistance to ciprofloxacin increased from 15% to >50% over the last 4 years. Resistance to gentamicin ranged between 20% and 40%. Staphylococci remained uniformly susceptible to vancomycin. There was no trend of increasing resistance among Staphylococcal species to semisynthetic penicillins, but 15% of *S. aureus* and 80% of CoNS were resistant to methicillin over the duration.

Gram-negative organisms. In the *Enterobacter*, *Citrobacter*, *Serratia* group, 40%–45% of the pathogens were resistant to ceftazidime and piperacillin, but none were resistant to the quinolones, carbapenems, and TMP-SMX. *E. coli* and *Klebsiella* species remained fully sensitive to most antibiotics except for resistance to piperacillin and TMP-SMX in 50% and 20% of these organisms. *P. aeruginosa* remained >90% sensitive to many agents, including imipenem. Exceptions were 15% re-
Figure 3. Resistance rates of *Streptococcus* species to specific antibiotics during the years shown. Numbers of strains tested given in parentheses. Cipro, ciprofloxacin; Genta, gentamicin; Imip, imipenem; Pen, penicillin; spp, species.

Antibiotic Usage

Empiric antibiotic usage has varied over the years. Cefazidime was the drug most commonly used for empiric therapy. The amount used diminished dramatically on a per-patient basis. The use of imipenem has remained constant over the 7-year period, although, overall, 14% of the patients received imipenem either as the initial drug or as a modification of cefazidime therapy. TMP-SMX was the most commonly used for prophylaxis early in the study period before the initial episode of febrile neutropenia but was superseded by other agents for antibacterial prophylaxis and then used for *Pneumocystis* prophylaxis after transplantation. Ciprofloxacin use began in earnest about 1994. The amount of ciprofloxacin used per patient in 1996 and 1997 was 2- and 4-fold the amount used in 1993–1995. β-lactams began to be used more frequently in 1994 as prophylaxis against gram-positive organisms or for concern about line infections before the neutropenic period and were continued until patients became febrile. This usage reached a peak of 57% of patients in 1997 (figure 4) Similarly macrolide prophylaxis directed against streptococci began to increase in 1995 and reached 30% of patients in 1997 (figure 4).

DISCUSSION

The trends in the organisms isolated from patients with neutropenia and fever have been reported from the European Organization for Research and Treatment of Cancer trials [8, 12]. Few authors have addressed trends in susceptibility in hematology units [13], and to our knowledge no large review has been published specifically addressing the changes in susceptibility pattern in BMT recipients. Unlike other institutions [14], our unit witnessed a yearly decrease in incidence of patients with bacteremias. In 1991, 56% of BMT recipients had ≥1 positive blood culture. This dropped to ~20% by 1997. The reduction in bacteremia is most striking for primary infections with gram-positive pathogens (figure 2), but primary gram-negative isolates also fell. Similar to non-BMT neutropenic patients, gram-positive blood isolates surpassed gram-negative isolates for each year [13–16], but this trend may be reversing, because the ratio of gram-positive to gram-negative pathogens has gradually declined from 2.7 to 1.3 (figure 1). There may be several possible explanations for these trends. Since 1994, there has been a dedicated team for the insertion of central lines, which has lowered the line infection rate. In about 1994 and most pronounced in 1996 prophylaxis directed against gram-positive organisms and gram-negative organisms, using β-lactams, macrolides, and quinolones, was more frequently used. Thus, the fall in gram-positive infections may be explained by these specific actions. The reduction in gram-negative organisms is probably due to ciprofloxacin use [17, 18].

Among gram-positive organisms, *Streptococcus* species remained the most frequently isolated pathogen. Viridans streptococcal bacteremia has been associated with the type α *Streptococcus* shock syndrome in 3%–33% of patients with a 6%–30% mortality rate [19, 20]. In our patients, however, no direct mortality (i.e., with a 7-day period) could be attributed to *Streptococcus* species bacteremia. A low rate or absence of mortality has also been reported in the nontransplant neutropenic population [11, 14, 21, 22]. The prompt institution of broad-spectrum antimicrobial agents at the onset of fever or the prompt addition of vancomycin for the report of a gram-positive may have limited the progression of bacteremia into a shock syndrome despite the high incidence of resistance to penicillin in the later years. The 8% mortality rate associated with CoNS bacteremia is similar to the 7% rate reported by others [23]. None of the 12 patients with *S. aureus* bacteremia died. This absence of mortality is in contrast with 6%–22% mortality rates in non-neutropenic patients [24, 25]. This favorable outcome may be due to early empiric treatment or perhaps the absence of a deleterious host-pathogen interaction mediated by neutrophils.

*E. coli* and *Klebsiella* species remained the most common
causes of gram-negative bacteremia but were associated with a low mortality (3%). Although *P. aeruginosa* accounted for only 14% of the gram-negative bacteremias, it was the pathogen with the highest mortality (40%), despite adequate empiric antibiotic coverage in all cases.

The development of resistance to antimicrobial agents is of growing concern in the hospitalized patient population as a whole and is well recognized in the febrile neutropenic patient [9]. The most striking example of the development of resistance in our patients is shown in figure 3. *Streptococcus* species, showed a substantial increase in penicillin resistance, from <10% to >50% of tested isolates over a 7-year period. This trend has been noted in general populations of neutropenic patients. [9, 26–28]. Although we did not routinely test susceptibility to ceftazidime, it can be assumed that an equal or greater proportion of these isolates would also be resistant to this agent [26, 27, 29]. Noteworthy is the emerging resistance to imipenem. Prior to 1996, no resistance was documented. In the 1996–1997 calendar years, 25% of tested isolates displayed resistance. Other authors have reported complete susceptibility to this agent [26, 30, 31]. We cannot explain this increase with the increased use of this agent over time, because this did not occur and only 14% of our patients received imipenem. We speculate that this is part of the increasing resistance that is seen among streptococcal species with attention being paid primarily to *S. pneumoniae*. If antibiotic use in our patients played a part, then it is likely that the high incidence of β-lactam prophylaxis may be implicated. This may have resulted in *S. pneumoniae*.

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Although prophylaxis may have been a contributing factor, this trend began earlier than the 1996 and 1997 years when ciprofloxacin, β-lactam, or macrolide prophylaxis were used more extensively. The accepted trend in the switch to gram-positive organisms in febrile neutropenic patients appears to be reversing, and, in the period following this review, this continues in neutropenic patients as a whole in our institution (authors’ unpublished data). Gram-negative bacteremia, especially due to *P. aeruginosa*, continues to have the highest mortality rate. Unlike many published reports, the most common cause of bacteremia, *Streptococcus* species did not result in any mortality within 7 days. Resistance trends in this area are of concern with resistance to ceftazidime in *P. aeruginosa*, the Bush group I β-lactamase–producing organisms, and *Streptococcus* species. As these trends become more widespread, empiric therapy, whether monotherapy or combination, will require changes designed to cover these specific problems. The recommendation of ceftazidime as a first line agent in the Infectious Diseases Society of America guidelines for the empiric treatment of febrile neutropenic patients [35] may not be appropriate for institutions with high incidence of streptococcal infections, because penicillin resistance is likely to be widespread [29] and not limited to our institution. In addition, there needs to some concern about the use of other β-lactams, because some amount of cross-resistance will occur, as we have seen with imipenem.

References


