Antibiotic-Resistant Bacteria in Surveillance Stool Cultures of Patients with Prolonged Neutropenia

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The value of stool surveillance for antibiotic-resistant gram-negative bacteria was analyzed in 86 neutropenic bone marrow transplant patients. Twice-weekly specimens were inoculated onto culture medium containing gentamicin plus carbenicillin. The recovered organisms were identified to the species level and tested for antibiotic susceptibility. Forty-eight resistant organisms were recovered from 35 patients. Thirteen isolates persistently colonized patients. Escherichia coli (29%) and Pseudomonas aeruginosa (19%) were the most frequently recovered organisms. Although most organisms were recovered while patients were on antibiotics, 15 isolates, including eight of nine resistant P. aeruginosa, were detected before antibiotics were initiated. The duration of antibiotic use was longer for patients persistently colonized than for those not colonized (P = 0.03). Of the 15 resistant organisms which caused infection, 12 were detected in the surveillance cultures. Infections by antibiotic-resistant organisms occurred more frequently in patients colonized than in those not colonized (P = 0.006) and more frequently in patients persistently colonized than in those colonized only once (P = 0.01). The absence of colonization or persistent colonization correlated well with the absence of infection (negative predictive values of 94 and 91%, respectively).

Neutropenic patients are especially prone to life-threatening infection due to gram-negative bacteria. Because potential gram-negative pathogens are present in the gastrointestinal tracts of these patients, a number of studies have examined the utility of stool surveillance cultures in predicting infections in neutropenic patients (1-5, 7, 8, 10, 12-15). However, identification of all organisms present on routine surveillance cultures not only is not feasible for clinical laboratories but also has been proven of limited clinical value.

With the routine use of empiric broad-spectrum antibiotics for fever during neutropenia, the emergence of antibiotic-resistant gram-negative bacteria as potential pathogens has become an increasing problem in recent years. One previous study considered the significance of antibiotic-resistant Pseudomonas aeruginosa in stool surveillance cultures (7) and found that resistant variants may be less virulent than susceptible strains.

A program of stool surveillance cultures in bone marrow transplant (BMT) patients has been in effect at this institution to alert clinicians to potential pathogens which would not be covered by the standard empirically derived therapeutic protocols. Patients were monitored during a cytotoxic therapy that was expected to result in neutropenia exceeding 14 days in duration. Our surveillance program was designed to detect carbenicillin and aminoglycoside-resistant organisms colonizing the intestinal tract. This study is an evaluation of the information gained from that program.

MATERIALS AND METHODS

Patients studied. Eighty-six consecutively treated patients underwent allogeneic BMT with marrow from genotypically HLA-identical siblings as treatment for leukemia or aplastic anemia over a 2-year period. Patients with leukemia were either in complete remission or early bone marrow relapse.

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antly with the institution of antibiotics. If fever persisted for >72 h after the institution of antibiotics, the aminoglycoside was generally discontinued, and trimethoprim-sulfamethoxazole was substituted. If an additional 72 h passed without defervescence, an empiric trial of amphotericin B was usually instituted. Once instituted, antibiotics were then continued until granulocyte recovery and modified according to the schema described above or as dictated by cultures and clinical state.

Surveillance cultures. Stool or rectal swab specimens were screened twice weekly for antibiotic-resistant gram-negative bacteria. Stool specimens were plated on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.), on agar containing 10 µg of gentamicin per ml and 250 µg of carbenicillin per ml, and on Pseudomonas agar base with Pseudo C-N supplement (Oxoid USA, Inc., Columbia, Md.). Any morphologically distinct colonies grown on these screening media at 24 h were picked and identified to the species level for further accurate susceptibility testing by the standard agar dilution method (11). Ticarcillin was used in place of carbenicillin at this second stage of testing. Isolates were judged to be resistant if the MIC of gentamicin or tobramycin exceeded 4 µg/ml and the MIC of ticarcillin exceeded 32 µg/ml. There was generally no greater than one twofold antibiotic dilution difference between the MICs of gentamicin and tobramycin. Organisms resistant to ticarcillin and gentamicin will be referred to as antibiotic resistant hereafter. Isolates recovered from two or more samples from a given patient were considered persistent colonizers of that patient. Organisms were considered susceptible to tetracycline (Tet⁴), chloramphenicol (Chl¹), cephalothin (Cep¹), amikacin (Ami⁴), piperacillin (Pip⁴), sulfoxadiazin sulfate (Sul⁺), trimethoprim-sulfamethoxazole (T-S), cefotaxime (Ctx¹), and moxalactam (Mox¹) at MICs of less than or equal to 4, 8, 8, 8, 64, 32, 1.6 and 32, 8, and 8 µg/ml, respectively.

Criteria for association with infection. An isolate was considered to be the cause of infection if the organism was recovered from the blood or from a localized site of infection.

Analysis. Predictive values were calculated according to the methods of Galen and Gambino (6) and are defined as follows. The positive predictive value is the percentage of patients with positive surveillance culture results who developed infection and was calculated as (true positives/true plus false positives) × 100, where the true positives are the number of patients with positive surveillance culture results and infection, and the true plus false positives are the total number of patients with positive culture results. The negative predictive value is the correlation of negative surveillance cultures with the absence of infection and was calculated as (true negatives/true plus false negatives) × 100, where the true negatives are the number of patients with negative culture results and without disease, and the true plus false negatives are the total number of patients with negative culture results. The sensitivity is the percentage of patients with infection who had positive surveillance cultures and was calculated as (true positives/true positives plus false negatives) × 100, where the true positives are as stated above, and the true positives plus false negatives are the sum of all patients with infection. The specificity is the percentage of patients without infection who had negative test results and was calculated as (true negatives/true negatives plus false positives) × 100, where the true negatives are as stated above and the true negatives plus false positives are the total number of all patients without infection. Patients colonized or infected by more than one antibiotic-resistant organism were counted once for each organism in these calculations. Other data were analyzed by the Fisher exact test or the Student t test as computed by Epistat, a computer software program.

RESULTS

Antibiotic-resistant bacteria in stool specimens. No antibiotic-resistant organisms were isolated from the stool specimens of 51 patients. Forty-eight antibiotic-resistant organisms were isolated from the stool specimens of 35 of 86 patients (Table 1). Thirteen of these organisms were present on multiple surveillance cultures and were termed persistent colonizers. Escherichia coli and P. aeruginosa were the most frequent antibiotic-resistant organisms isolated, and together they accounted for 48% of all resistant organisms recovered. Although most organisms were transient colonizers, Enterobacter cloacae isolates were persistently recovered from stool specimens in all four occurrences of the organism. The first appearance of E. cloacae always occurred late in the course of antibiotic therapy (mean, 11.8 days after the start of antibiotics).

Time of first appearance. P. aeruginosa and E. coli were early colonizers (means, −7.7 and 2.4 days, respectively, after the initiation of antibiotics) (Table 1). P. aeruginosa appeared earlier than did E. coli (P = 0.009), E. cloacae (P = 0.002), Klebsiella oxytoca (P = 0.01), Citrobacter freundii (P = 0.005), and Klebsiella pneumoniae (P = 0.02). E. coli appeared earlier than did E. cloacae (P = 0.004), C. freundii (P = 0.05), K. pneumoniae (P = 0.008), and K. oxytoca (P = 0.01). Fifteen antibiotic-resistant isolates were recovered from stool specimens before the initiation of antibiotics (Table 1). Although for most organisms isolation before the start of antibiotics was uncommon, eight of the nine antibiotic-resistant P. aeruginosa isolates were present before the initiation of antibiotics. The likelihood of recovery before antibiotic use was greater for the P. aeruginosa isolates (8 of 9) than for the other organisms combined (7 of 39) (P = 0.0001). Of the 15 isolates present before antibiotic use, only 3 were present in the first stool specimen obtained. The other 12 were first detected after an average of 2.3 negative stool cultures.

Relationship of the presence of antibiotic-resistant bacteria in stool specimens to duration of antibiotic use. The duration of antibiotic use was not significantly longer among patients

| Table 1: Gram-negative bacteria resistant to gentamicin and ticarcillin recovered from the stool specimens of 86 consecutive allogeneic BMT patients (48 isolates in 35 patients) |
|---------------------------------|-----------------|-----------------|-----------------|
| Organism (no. of isolates)      | No. of persistent colonizers* | First appearance of isolate (days)* | No. (%) of isolates present before antibiotics |
| Escherichia coli (14)           | 4                | 2.4             | 3 (14)          |
| Pseudomonas aeruginosa (9)      | 1                | −7.7            | 8 (89)          |
| Citrobacter freundii (6)        | 1                | 9.7             | 1 (17)          |
| Enterobacter cloacae (4)        | 4                | 11.8            | 0 (0)           |
| Klebsiella oxytoca (4)          | 1                | 23.2            | 1 (25)          |
| Serratia marcescens (3)         | 1                | 1.0             | 1 (33)          |
| Klebsiella pneumoniae (3)       | 1                | 26.3            | 0 (0)           |
| Pseudomonas putida (3)          | 0                | 1.7             | 2 (33)          |
| Pseudomonas acidovorans (1)     | 0                | 2               | 0 (0)           |
| Enterobacter aerogenes (1)      | 0                | 28              | 0 (0)           |

* Present on two or more samples.

b Mean number of days after initiation of antibiotics.
colonized with resistant organisms than it was among those not colonized (25 and 16 days, respectively; \( P = 0.21 \)). Among patients persistently colonized, antibiotic use was significantly longer than it was among those not colonized (40 and 16 days, respectively; \( P = 0.03 \)).

**Susceptibility to other antibiotics.** The susceptibility of the 48 stool surveillance culture isolates is shown in Table 2. All isolates were resistant to ampicillin, ticarcillin, gentamicin, and tobramycin, and these MICs are not included in the table. Of the antibiotics tested, cefotaxime and moxalactam demonstrated the best in vitro activity against the surveillance culture isolates, although they had no activity against any of the *P. aeruginosa* isolates.

**Recovery of bacteria not resistant to both carbenicillin and gentamicin.** Nine isolates (from nine patients) were recovered from the screening plates but did not prove to be resistant to both ticarcillin and gentamicin on subsequent confirmatory testing. Five isolates were resistant to ticarcillin but not to gentamicin (two *E. coli* and *K. oxytoca* isolates each and one *C. freundii* isolate), three *P. aeruginosa* isolates were susceptible to both ticarcillin and gentamicin, and one *P. aeruginosa* isolate was resistant to gentamicin but susceptible to ticarcillin. None of these isolates were associated with infection.

**Infections due to antibiotic-resistant bacteria.** Fifteen antibiotic-resistant organisms caused 13 infections. There were eight perianal infections, one perianal infection with bacteremia, and four episodes (caused by six organisms) of bacteremia unassociated with localized infection. Twelve of these organisms were present in the stool surveillance cultures.

**TABLE 3.** Association of colonization and infection due to antibiotic-resistant bacteria in 86 consecutive allogeneic BMT patients

<table>
<thead>
<tr>
<th>Colonization</th>
<th>No. of patients</th>
<th>Infected</th>
<th>Noninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients colonizedb</td>
<td>12</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>5</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Persistently'</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Patients not colonized</td>
<td>3</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a} \) Patients colonized by more than one antibiotic-resistant organism were counted once for each organism.

\( ^{b} \) Patients colonized at least once were more likely to become infected than those not colonized (\( P = 0.006 \)).

\( ^{c} \) Patients persistently colonized were more likely to become infected than those colonized only once (\( P = 0.01 \)).

Three organisms (which caused two episodes of bacteremia, one of which was in combination with an organism that was detected in the stool surveillance cultures) were not detected in the stool cultures; these occurred in patients colonized by other antibiotic-resistant organisms. None of the 51 patients whose stool cultures were free of the antibiotic-resistant organisms developed infection due to antibiotic-resistant organisms. These data are given in Table 3, where patients (and infections) are counted once for each unique organism encountered, as described in Materials and Methods. Data comparing persistently colonized patients with those not persistently colonized are also given in Table 3. Infection due to antibiotic-resistant organisms occurred more frequently among patients colonized by these organisms (12 of 48 patients) than among patients not colonized (3 of 54 patients) (\( P = 0.006 \)). Although infection occurred in 7 of 13 persistently colonized patients, infection occurred in only 5 of 35 patients colonized only once (\( P = 0.01 \)).

The sensitivity, specificity, positive predictive values, and negative predictive values are given in Table 4 for colonization and persistent colonization. Persistent colonization had greater specificity and a greater positive predictive value than colonization, but it had a lower sensitivity. The negative predictive values were high for both colonization and persistent colonization.

**TABLE 4.** Ability of surveillance cultures to predict infection due to antibiotic-resistant bacteria in 86 consecutive allogeneic BMT patients

<table>
<thead>
<tr>
<th>Colonization</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent colonization</td>
<td>12/15 (80%)</td>
<td>51/87 (59%)</td>
<td>12/48 (25%)</td>
<td>51/54 (94%)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>7/15 (47%)</td>
<td>81/87 (93%)</td>
<td>7/13 (54%)</td>
<td>81/89 (91%)</td>
</tr>
</tbody>
</table>

\( ^{a} \) Patients colonized by more than one antibiotic-resistant organism were counted once for each organism. Predictive values are defined in the text.
Twenty-one infections due to gram-positive organisms and two infections due to fungi were also noted in the 86 patients.

DISCUSSION

Neutropenic patients are extremely susceptible to morbidity and mortality from infection due to gram-negative bacteria. Early empiric antibiotic use has been shown to reduce morbidity and mortality. Because most neutropenic patients are exposed to antibiotics, the risk of developing infections from antibiotic-resistant organisms is substantial. In addition, it is critical that neutropenic patients be placed on the appropriate antibiotics promptly when infection occurs. Therefore, identification of neutropenic patients at high risk of infection due to antibiotic-resistant bacteria is a high priority.

Studies by surveillance cultures of the bacterial flora of neutropenic patients have been done previously (1–5, 7, 8, 10, 12–15). These studies were not designed to detect resistant strains but rather to examine the significance of isolates present on media that contained no antibiotics. In one study Schimpff et al. (14) found that colonization by P. aeruginosa was associated with sepsis in most neutropenic patients. After improvements in hygienic measures and changes in oral antibiotics, this association decreased substantially (13). Gurwith et al. (8) found that 20 of 42 evaluable bacteremic episodes had antecedent positive surveillance cultures. However, in 14 of 42 episodes, there was no correlation between surveillance and bacteremic organisms. Fainstein et al. (5) noted that changes in throat and stool flora occurred during hospitalization; although organisms acquired during hospitalization were more apt to cause infection, most colonizing organisms did not cause infection.

A program of identification of all organisms present on surveillance cultures as performed in these earlier investigations is not feasible for routine use in most hospital laboratories and has been proven in these earlier studies to be of limited clinical value. To minimize the cost and labor, we instituted a program to identify only those organisms which were resistant to the antibiotics employed in our empiric regimen, i.e., ticarcillin plus gentamicin or tobramycin. Our hypothesis was that a failure to detect antibiotic-susceptible organisms would have minimal clinical impact, since signs and symptoms of sepsis would lead to the institution of antibiotics active against the organisms and to the subsequent control of the infection. Our findings in this study support this contention, since susceptible bacteria caused sepsis only in patients not on antibiotics, and these episodes were readily controlled by the institution of antibiotics.

The patients in this study were found to frequently harbor resistant bacteria. Almost a third of the organisms were present before the institution of antibiotics. P. aeruginosa was especially apt to colonize patients before antibiotic use. Of the 15 isolates recovered before antibiotic use, only 3 were present in the first stool specimen obtained. The other 12 isolates first appeared after an average of more than 1 week of negative cultures. This suggests hospital acquisition, but variability in the sampling makes this supposition necessarily tentative.

Greene et al. (7) found that the P. aeruginosa variants that were resistant to both carbenicillin and gentamicin appeared to be less virulent than did the susceptible strains. Although our study was not designed to compare virulence of susceptible and resistant bacterial variants, our finding that only susceptible organisms caused bacteremia in patients not receiving antibiotics is compatible with the findings of Greene et al. that susceptible bacteria have a competitive advantage over resistant bacteria. Alternatively, titers of resistant organisms could have been lower than those of susceptible organisms, giving the resistant organisms a competitive disadvantage. Only 12% of the P. aeruginosa isolates recovered from our patients caused infection in our study. In contrast, 29% of the resistant E. coli isolates caused infection. This may have been due to the greater persistence of the E. coli isolates or to the difference in the number of organisms. The disparity may also suggest that the resistant P. aeruginosa variants are less virulent than are the resistant E. coli variants.

Because the antibiotic protocol that was used for these patients was instituted promptly for fever and modified by knowledge of the stool surveillance cultures, positive blood cultures and localized sites of infections were infrequently documented. For this reason, conclusions regarding the helpfulness of the surveillance program necessarily underestimate the ability of the surveillance program to predict infection. Only documented infections were included in the analysis. Thirteen febrile patients from whom bacteria were not recovered from blood and in whom no local site of infection was documented showed clinical response only when antibiotics were modified appropriately in response to the organisms recovered on surveillance cultures.

Although the sensitivity of overall colonization was good, the specificity was much lower, and the positive predictive value was poor. Many patients colonized with resistant organisms developed no infection from these organisms. When persistent colonization alone was considered, the sensitivity and positive predictive value were low, but the specificity was good. The negative predictive values of both overall and persistent colonization were excellent: negative cultures predicted absence of infection to a high degree. Thus, in this group of patients with prolonged neutropenia caused by cytotoxic therapy (which also damaged the gastrointestinal mucosal integrity), surveillance to detect antibiotic-resistant bacteria was helpful in identifying patients at low risk for developing antibiotic-resistant bacterial infection, although surveillance was somewhat less helpful in identifying those at high risk. Three organisms implicated in two bacteremic episodes were not detected. Thus, continued study is required to optimize the value of such surveillance culture programs and to determine the patient populations for which this approach has greatest value.

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LITERATURE CITED


