Cefepime-resistant Gram-negative bacteremia in febrile neutropenic patients with hematological malignancies

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SUMMARY

Objectives: This study was performed to determine the local etiologic pattern of blood culture isolates and antibiotic resistance in febrile neutropenic patients with hematological malignancies.

Methods: A total of 142 blood culture isolates from febrile neutropenic patients admitted to our hematology unit were examined, particularly for the detection of cefepime resistance, because cefepime, a fourth-generation cephalosporin, has been used in our unit as initial therapy for febrile neutropenia.

Results: Among all isolates, 67 (47.2\%) were Gram-positive bacteria, the majority of which were fully sensitive to vancomycin. Gram-negative bacteria accounted for 68 (47.9\%) of the isolates. Cefepime resistance was seen in 24 (35.3\%) of the Gram-negative isolates, and had significantly increased in 2007. The cefepime-resistant isolates primarily consisted of \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, and \textit{Klebsiella pneumoniae}. Approximately 60\% of the cefepime-resistant isolates were extended-spectrum \(\beta\)-lactamase (ESBL)-producing organisms. Molecular analysis showed the predominant emergence of CTX-M types. Most of the cefepime-resistant isolates were resistant to third- and various fourth-generation cephalosporins, while having a high susceptibility to carbapenems, particularly meropenem.

Conclusions: Cefepime resistance was often detected in the blood culture isolates from febrile neutropenic patients. This result suggests that therapeutic strategies for febrile neutropenia should be modified based on the local antibiotic resistance patterns.

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1. Introduction

Bacteremia is clinically seen in febrile neutropenic patients with hematological malignancies. In particular, Gram-negative organisms are renowned for inducing septic shock, which is often followed by sepsis-related death. Several groups have reported that the prevalence of Gram-negative bacteremia has increased among episodes of febrile neutropenia in hemato-oncology units.\textsuperscript{1,2} A prompt initiation of empirical antibiotic therapy is favorable for patients with febrile neutropenia, regardless of the detection of bacteremia. Many clinical studies have recommended broad-spectrum antibiotics covering both Gram-positive and-negative bacteria as first-line treatment for febrile neutropenic patients. Certain specific agents have been used worldwide, including third- and fourth-generation cephalosporins, carbapenems, and \(\beta\)-lactam/\(\beta\)-lactamase inhibitor combinations. In recent years, monotherapy using these antibiotic agents has been shown to be comparable to combination therapy with regard to the efficacy of treatment of febrile neutropenia.\textsuperscript{3–6} Based on these studies and guidelines,\textsuperscript{7,8} our unit adopted the single administration of cefepime, a fourth-generation cephalosporin, as the initial treatment for febrile episodes in neutropenic patients. It is now unquestioned that the use of broad-spectrum antimicrobial agents renders many clinically important organisms highly resistant to various antibiotics. In the general population, extended-spectrum \(\beta\)-lactamase (ESBL)-producing Gram-negative bacteria have been reported to be increasing in prevalence among all bacteria detected, and an increase in the use of cephalosporins may be related to the emergence of ESBL-producing bacteria.\textsuperscript{9,10} In febrile neutropenic patients with hematological malignancies, the relatively recent resistance pattern of Gram-negative bacteremia appears inconclusive. Commonly used agents, such as cefepime and carbapenems, have shown high activity against Gram-negative bacteremia detected in neutropenic patients,\textsuperscript{11} while other studies have reported the occurrence of Gram-negative bacteremia resistant to many antibiotics.\textsuperscript{12–14} The susceptibility pattern of organisms isolated from one region may not be the same as that found in other regions of the world, as various environmental...
Febrile neutropenia was defined as a neutrophil count below 1000 cells/l and an axillary temperature of >38.0 °C. Cefepime, a fourth-generation cephalosporin, has been adopted as an initial empirical antibiotic agent for febrile neutropenic patients. Cefepime at a dosage of 4 g/day was used, and during the study period the defined daily dose per 1000-patient days of cefepime was 171. The administration of antibiotics was continued until recovery of neutrophil counts and/or resolution of infection. When bacteria were isolated from a blood culture, antimicrobial therapy was adjusted according to the antibiotic resistance patterns obtained.

2. Methods

2.1. Patients

A total of 872 patients were admitted to the blood and marrow transplantation unit at Hara-Sanshin Hospital from January 2006 to December 2008. Bacteremia in febrile neutropenic patients was analyzed in the present study for two reasons: (1) the number of Gram-negative strains isolated up to the year 2005 was very small, and (2) some of the patients admitted up until 2005 had received prophylactic administration of antibiotics during neutropenic periods. The prophylactic use of antibiotics is known to affect the etiology of febrile neutropenic bacteremia. Neutropenic patients admitted after January 2006 had only taken antimycotic agents for prophylaxis.

All the patients with febrile neutropenia were registered and their informed consent for study participation was obtained. Febrile neutropenia was defined as a neutrophil count of < 0.5 × 10^9 cells/l and an axillary temperature of >38.0 °C. Detailed information on patients was collected from computer databases. These data included age, sex, malignant disease classification, presence of indwelling catheter, neutrophil counts, prior antibiotic usage, and clinical outcome. Information on isolated strains, including etiology and susceptibility to antibiotics, was obtained from a microbiology laboratory computer database.

2.2. Microbiology

In our unit, blood culture tests are conducted for all patients with febrile neutropenia. An automated blood culture system (BACTEC) was used for each test. Several samples were obtained from the same patient and treated as independent results. Antibiotic susceptibilities were determined by the breakpoints standardized by the Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS). The screening and confirmation tests for ESBL and metallo-β-lactamase were conducted according to the recommendations of the CLSI. In addition, β-lactamase producers were confirmed using a Cica β test I/MBL kit (Kanto Chemical Co. Ltd, Tokyo, Japan). Strains that were positive for ESBL based on the confirmation tests were then evaluated for genotype. The genotyping of metallo-β-lactamase was conducted when the screening test was positive. PCR was conducted using five sets of primers to amplify type-specific ESBL genes, including CTX-M, TEM, and SHV types. Three sets of primers were used to detect group-specific CTX-M β-lactamase genes, and then the different types of CTX-M were determined by DNA sequencing. PCR was conducted using primers specific for metallo-β-lactamase genes.

2.3. Statistical analysis

For the basic characteristics of patients with cefepime-resistant isolates and patients with cefepime-sensitive isolates, categorical variables were analyzed using a two-tailed Chi-square test, and continuous variables were compared using the Mann–Whitney U-test. Odds ratios and 95% confidence intervals were calculated to compare resistance patterns to various antibiotics between cefepime-resistant and cefepime-sensitive isolates. A value of \( p < 0.05 \) was considered to be statistically significant. All statistical calculations were performed using SAS software (SAS Institute, Inc., Cary, NC, USA).

Table 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>26</td>
<td>18.3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>21</td>
<td>14.8</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13</td>
<td>9.2</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>5</td>
<td>3.5</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>Gram-negative, total</td>
<td>68</td>
<td>47.9</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus species, total</td>
<td>47</td>
<td>33.1</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>46</td>
<td>32.4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Streptococcus species, total</td>
<td>9</td>
<td>6.3</td>
</tr>
<tr>
<td>α-Hemolytic streptococci</td>
<td>6</td>
<td>4.2</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>Enterococcus species</td>
<td>8</td>
<td>5.6</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>Gram-positive, total</td>
<td>67</td>
<td>47.2</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>4.9</td>
</tr>
<tr>
<td>Isolates, total</td>
<td>142</td>
<td>100</td>
</tr>
</tbody>
</table>
The prevalence of Gram-negative isolates resistant to cefepime, an antibiotic agent adopted at our institution as an initial therapy for febrile neutropenia, was examined because a high rate of resistance to cefepime affects the choice of empirical antibiotics for febrile neutropenia. Table 2 shows the number of cefepime-resistant Gram-negative strains isolated from febrile neutropenic patients in each year of this study. Cefepime-resistant isolates comprised 24 (35.3%) of 68 Gram-negative isolates analyzed, and had significantly increased in 2007. About half of the isolates of *E. coli* and *P. aeruginosa* showed resistance to cefepime, and approximately 20% of *K. pneumoniae* were resistant to cefepime.

### 3.2. Phenotypic and molecular characterization of cefepime-resistant Gram-negative strains

The cefepime-resistant Gram-negative isolates were then characterized with respect to production of β-lactamase enzymes. Screening of strains producing ESBL and metallo-β-lactamase was conducted. Four *P. aeruginosa* isolates were suspected to be metallo-β-lactamase producers, but no strains were confirmed as metallo-β-lactamase producers using PCR methods. All cefepime-resistant *E. coli* and *K. pneumoniae* strains were phenotypically identified as ESBL-producing isolates by the clavulanate test, and all were confirmed as isolates containing ESBL genes at a molecular level. Table 3 shows the ESBL genotypes of all cefepime-resistant *E. coli* and *K. pneumoniae* strains. Twelve cefepime-resistant *E. coli* isolates were classified as the following four types: CTX-M-14 alone, a combination of CTX-M-14 and CTX-M-2, a combination of CTX-M-14 and TEM, and TEM alone. Two *K. pneumoniae* strains resistant to cefepime produced CTX-M-14 and SHV-β-lactamase. Although there is concern over the spread of a particular ESBL strain through nosocomial outbreaks, the detection of different ESBL genotypes indicates that these ESBL-producing strains did not disseminate from a single strain with a certain genotype.

### 3.3. Antimicrobial susceptibilities of cefepime-resistant Gram-negative strains

Table 4 shows the resistance rates to different antibiotics of cefepime-resistant, ESBL-producing, and cefepime-sensitive isolates. Most of the cefepime-resistant and ESBL-producing isolates were resistant to all generations of cephalosporins, and this resistance pattern was significantly different to that of cefepime-sensitive isolates. Most cefepime-resistant isolates were resistant to various fourth-generation cephalosporins, such as cefozopran and cefpirome. In contrast, most cefepime-resistant isolates retained favorable susceptibility to carbenapenems, and only approximately 10% of cefepime-resistant isolates were resistant to meropenem. All ESBL-producing isolates were sensitive to carbenapenems. Most of the cefepime-resistant and ESBL-producing isolates were susceptible to aminoglycosides.

Serial blood cultures were acquired for 20 of 24 patients (83.3%) with cefepime-resistant, 12 of 14 patients (85.7%) with ESBL-producing, and 39 of 44 patients (88.6%) with cefepime-sensitive bacteremia. The detected bacteria were eliminated after antibiotic treatment in all of the groups. In the control patients with cefepime-sensitive bacteremia, no patients died of infection during antibiotic therapy. In case patients with cefepime-resistant bacteremia, one patient died during treatment, due to possible septic shock caused by *P. aeruginosa* This *P. aeruginosa* isolate was resistant to carbenapenems, including imipenem/cilastatin and meropenem. There were no mortalities among patients with ESBL-producing bacteremia during antimicrobial therapy.

### 4. Discussion

The present study results indicate an increase in cefepime-resistant Gram-negative bacteremia in febrile neutropenic patients with hematological malignancies under the therapeutic strategy of cefepime usage as an initial antibiotic treatment. Cefepime-resistant isolates consisted of *P. aeruginosa*, *E. coli*, and *K. pneumoniae*. None of the *P. aeruginosa* strains produced metallo-β-lactamase, while all of the *E. coli* and *K. pneumoniae* strains were ESBL producers. Furthermore, genotyping of the ESBL enzymes indicated the predominance of the CTX-M type. To our knowledge, no previous reports have described the molecular analysis of β-lactamase in the field of bacteremia recovered from febrile neutropenic patients. These data give rise to practical suggestions for surveillance and therapeutic strategies.

The present study indicated that CTX-M enzymes are most prevalent in ESBL-producing strains isolated from febrile neutropenic bacteremia. Recent surveillance tests have reported that organisms producing CTX-M β-lactamase, a new ESBL type, have been replacing those that produce TEM and SHV enzymes, the original ESBL types. CTX-M-producing bacteria acquire resistance to all generations of cephalosporins, while remaining highly susceptible to carbenapenems. The present data are consistent with this resistance pattern of organisms with CTX-M ESBL enzymes. As a characteristic of the CTX-M type, CTX-M-producing bacteria not
It would be reasonable to assume that Gram-negative bacteremia caused by febrile neutropenia could induce fatal infections, and that failure of an initial treatment for multidrug-resistant bacteremia might lead to a high mortality. However, in the present study, only one patient died during treatment of Gram-negative bacteremia, both cefepime-sensitive and cefepime-resistant. In most cases of patients with cefepime-resistant isolates, rapid bacterial detection and prompt antibiotic change resulted in successful treatment. All of the secondary antibiotics, except in two cases, were meropenem, and this treatment success clinically reinforces the finding that approximately 90% of the cefepime-resistant strains, including ESBL-producers, were sensitive to meropenem. The clinical outcome of infections caused by ESBL-producing organisms remains controversial, because no prospective studies have been conducted to assess prognosis in a statistically significant number of patients. All observations, including the present data, have been obtained from case-control studies with relatively small numbers of patients. A new study should be designed to specifically resolve this clinical question.

Our unit has faced an increase in bacteremia resistant to cefepime, which has been used as an initial antibiotic agent for febrile neutropenia, while carbapenems, especially meropenem, retain high activity against cefepime-resistant bacteria. Although it appears reasonable to choose carbapenems as the initial antimicrobial treatment for patients with febrile neutropenia, this treatment would be adopted only in the case of our patients. The present study identified a high prevalence of multidrug-resistant organisms with CTX-M ESBL types, which are currently on the increase worldwide. Identification of the genotypes of β-lactamase-producing organisms provides valuable information on antibiotic resistance patterns.

Conflict of interest

No conflict of interest to declare.

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References


