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ORIGINAL ARTICLE

Bloodstream infections in pediatric patients with acute leukemia: Emphasis on gram-negative bacteria infections

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KEYWORDS
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Abstract *Background/Purpose:* Acute leukemia is the most common pediatric hematological malignancy. Bloodstream infections (BSIs) are severe complications in these patients during chemotherapy. This study aims to explore clinical features, laboratory, and microbiological characteristics of BSIs in acute leukemic children.
Methods: Patients aged < 18 years, diagnosed with acute myeloid leukemia or acute lymphocytic leukemia with BSIs from January 2004 to December 2013 were enrolled. BSIs was defined as positive isolate(s) of blood culture and associated with clinical findings. Clinical presentations, demographic features, and microbiological findings were retrospectively reviewed.
Results: In total, 126 isolates of 115 episodes of BSIs were identified from 69 patients (acute lymphocytic leukemia 56; acute myeloid leukemia 13). Gram-negative bacteria (GNB), gram-positive cocci, and fungi constituted 56.3%, 42.3%, and 2.4% of the pathogens, respectively. Eighty-three and a half percent of BSIs occurred along with neutropenia, and 73% had severe neutropenia. GNB was the leading pathogen of BSIs. The major GNBs were *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. White blood cell counts, absolute neutrophil counts, and platelet counts were significantly lower in patients of BSIs caused by GNB than gram-positive cocci. Plasma level of C-reactive protein was significant high in patients of GNB BSIs (179.8 mg/L vs. 127.2 mg/L; $p = 0.005$). Eighty-two percent of patients of *E. coli*, *K. pneumonia*, and *P. aeruginosa* BSIs had sepsis related organ failure or organ dysfunction. *P.*

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aeruginosa BSIs had the highest case-mortality (40%).
Conclusion: Neutropenia was the major risk factor of BSIs in pediatric leukemic patients. BSIs of GNB were associated with severe neutropenia, systemic inflammatory responses, and high mortality.
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Introduction

Acute leukemia is the most common hematological malignancy in children, representing about 35% of all childhood cancers.[1] Advances in antimicrobial prophylaxis and chemotherapy have decreased the disease severity and improved the survival rate. Infectious complications are still the major causes of morbidity and mortality in hematological and oncological patients. Certain malignancies are inherently associated with immune deficits.[2] For example, patients with lymphocytic leukemia frequently have hypogammaglobulinemia leading to increased susceptibility to encapsulated bacteria. Such patients may have recurrent pulmonary infections and bacteremia. Patients with Hodgkin disease are at risk for opportunistic infections due to T-cell abnormalities.[3] However, most opportunistic infections occur during treatment with cytotoxic and immunosuppressive therapy.

Elevated quality of supportive care has permitted patients to recover successfully from the side effects of cytotoxic chemotherapy, radiation therapy, and intense immunosuppression agents. Nonetheless, the increasing use of novel aggressive chemotherapies and immunosuppressant agents, including biological response-modifying agents, has led to a growing population of patients with deficits in humoral- and cell-mediated immunity. Bacteria represent the immediate threat to most immunocompromised hosts. Bloodstream infections (BSIs) are still the cardinal infections through the course of chemotherapy in leukemic patients and may lead to fatality, even if antibacterial/antifungal prophylactic regimens have been administered.

To improve the care quality and survival of leukemia patients with BSIs, it is important to understand the risk factors for BSI during the course of chemotherapy for high-risk patients. The aims of this study were to explore the clinical manifestations, laboratory data, systemic complications, and microbiological characteristics of BSIs in pediatric leukemic patients.

Methods

Patients and setting

Patients aged $<$ 18 years, diagnosed with acute leukemia at the Department of Pediatrics, National Cheng Kung University Hospital, Tainan, Taiwan, from January 2004 to December 2013 were enrolled. The Institutional Review Board of National Cheng Kung University Hospital approved this study (/A-ER-103-406).

Definitions and laboratory analysis

Blood cultures were obtained routinely from acute leukemic patients with fever ($\geq$ 38°C) or hypothermia ($\leq$ 35°C), and in association with nonspecific signs and other organ or systemic symptoms. Respiratory symptoms (cough, rhinorrhea, and respiratory distress), gastrointestinal symptoms (nausea, vomiting, diarrhea, and abdominal pain), consciousness disturbance, mucositis, and rash were monitored. Other cutaneous signs of inflammation such as local heat, swelling, exudate, fluctuation, and ulceration were often diminished in neutropenic leukemia patients due to an impaired inflammatory response. At least one set of blood culture was obtained whenever infection was suspected, before empirical antibiotic treatment. All eligible patients were hospitalized when they met the criteria of the above-mentioned conditions. The following laboratory tests were performed: complete blood cell count, differential count of white blood cells (WBC), C-reactive protein (CRP), liver and renal function tests, chest X-ray, urinalysis, and urine and blood cultures.

BSI was defined as a patient having identified pathogen(s) from one or more blood cultures. All blood cultures were processed by the clinical microbiology laboratory using the Bactec 9240 system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Coagulase-negative staphylococci (CoNs) BSIs was defined as the same pathogens with the same antibiograms from at least two identified positive blood cultures. The definition of neutropenia is according to the number of patients' absolute neutrophil count (ANC). Neutropenia was defined as ANC $<$ 1500/μL. Mild neutropenia was ANC from 1000—1500/μL, moderate neutropenia was ANC from 500/μL to 1000/μL, and severe neutropenia was ANC $<$ 500/μL.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was determined using the disk diffusion technique. The zone diameters of each drug with the disk diffusion susceptibility method were interpreted using the criteria of the Clinical and Laboratory Standards Institute (CLSI), and categorical assignment was carried out by using CLSI breakpoints.[4] The following agents were tested: ertapenem, imipenem, meropenem, cefepime, ceftazidime, cefotaxime, piperacillin/tazobactam, ampicillin/sulbactam, ciprofloxacin, levofloxacin, gentamicin, amikacin, and flomoxef. Extended-spectrum β-lactamases (ESBL) production was screened and confirmed in all isolates by performing a double-disc synergy test of CLSI guidelines.[4] Among the *P. aeruginosa* isolates, extensively-drug resistant

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P. aeruginosa was defined as nonsusceptible to one or less agents in all but in two or more categories of antimicrobial agents, multidrug-resistant (MDR) *P. aeruginosa* was defined as nonsusceptible to one or more agents in three or more antimicrobial categories all antimicrobial agents, and pan drug-resistant *P. aeruginosa* was defined as nonsusceptible to all antimicrobial agents. The antimicrobial categories were including aminoglycosides, antipseudomonal carbapenems, antipseudomonal cephalosporins, antipseudomonal fluoroquinolones, antipseudomonal penicillins plus β-lactamase inhibitors, phosphonic acids, and polymyxins.[5]

Therapeutic regimens of antibiotics treatment

Antibacterials were initiated at the onset of fever as followings: in neutropenic patients, piperacillin/tazobactum plus amikacin (from June 2012 to December 2013) or cefazolin plus piperacillin plus gentamicin (from June 2004 to December 2012). In non-neutropenic patients, ampicillin/sulbactam or cefazolin plus gentamicin were administrated. If patients still had persistent fever after initiation of antibiotics for 3 days, or once fever subsided but infectious signs recurred subsequently under the same therapeutic regimens, the laboratory tests were reexamined and the original antimicrobial regimens were reevaluated.

Statistical analysis

The significance of differences in proportions data was determined using nonparametric statistics of two-tailed χ^2 test and Kruskal-Wallis H test. A p value < 0.05 was considered to be statistically significant. All the data were performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA).

Results

Demography data

Overall, 186 patients were diagnosis with acute leukemia, including 157 patients with acute lymphoblastic leukemia (ALL) and 29 with acute myeloid leukemia (AML). There were 115 episodes of BSIs that occurred in these patients and 126 isolates were identified. There were 93 episodes of BSIs in ALL patients, and 22 episodes of BSIs in AML patients. The men-to-women ratio was 1.67 (72:43), and the mean age was 9.2 ± 6.3 years. In ALL patients, the 1−5 years old group were the dominate age group (41.2%), but in AML patients the age distribution was average.

Clinical symptoms/signs and systemic complications

Only 2.6% of patients had underlying disease. The underlying diseases included congenital heart disease, mental retardation, Down syndrome, and chronic inflammatory demyelinating polyneuropathy. The most common clinical symptom was fever (95.7%) followed by respiratory symptoms (9.6%), mucosa damage (6.1%), gastrointestinal symptoms (6.1%), consciousness disturbance (1.7%), and rash (1.7%). Thirty-five cases (30.4%) had systemic complications (sepsis related organ failure or organ dysfunction), including shock/hypotension (24.3%), septic emboli (10%), acute renal function (5.6%), liver function impairment (4.3%), or central nervous system involvements (4.2%). The overall case fatality rate was 10.4% (12/115).

Laboratory and microbiological findings

Ninety-six (83.5%) episodes of BSIs occurred in association with neutropenia, and 73% patients had severe neutropenia. Laboratory data showed that the CRP level is significant higher in AML than ALL patients (191.9 mg/L vs. 146.8 mg/L; $p = 0.042$). WBC was significantly lower in ALL patients than AML (2300/mm^3 vs. 4400/mm^3; $p = 0.013$). Demographic data, clinical symptoms/signs, complications, and mortality did not show significant difference between ALL and AML patients.

Among gram-positive organisms of BSIs, CoNS (23.8%), *Streptococcus* spp. (7.1%), *Enterococcus* spp. (2.4%), and oxacillin-resistant *Staphylococcus aureus* (3.2%) were the major pathogens. As for the gram-negative organisms, *Escherichia coli* (22.2%), *Klebsiella pneumoniae* (13.5%), *Pseudomonas aeruginosa* (7.9%), and *Enterobacter* spp. (4%) dominated. Three episodes of fungal infections were noted, including *Candida albicans*, *Candida tropicalis*, and *Cryptococcus neoformans*, respectively (Table 1). There was no difference in the percentage of causative pathogens, gram-positive cocci and gram-negative bacteria (GNB), between AML and ALL patients.

In comparison with BSIs of gram-positive cocci, sixty-five (91.5%) GNB BSIs occurred in association with severe neutropenia. Patients with GNB BSIs had significantly lower WBC (3300/μL vs. 12,900/μL; $p = 0.001$), ANC (606/μL vs. 1256/μL; $p = 0.041$), and platelet counts (65 × 10^3/μL vs. 120 × 10^3/μL; $p = 0.039$); and significantly higher CRP level (179.3 mg/L vs. 127.2 mg/L; $p = 0.005$). Patients with GNB BSIs had more systemic complications of shock/hypotension. (32.4% vs. 9.6%; $p = 0.001$; Table 2).

In patients with GNB BSIs, the mean WBC were 1700/μL, 600/μL, and 700/μL in *E. coli*, *K. pneumoniae*, and *P. aeruginosa* BSIs patients, respectively. In comparison with patients of *E. coli* BSI, significantly lower WBC (700/μL vs. 1700/μL) and ANC (255/μL vs. 713/μL) were found in patients of *P. aeruginosa* BSIs ($p < 0.05$). The mean plasma level of CRP showed 218.6 mg/L, 208.5 mg/L, and 135.3 mg/L, respectively. The level of direct form bilirubin was significant higher in patients of *P. aeruginosa* BSIs than in patients of *E. coli* and *K. pneumoniae* BSI (7.5 mg/dL vs. 3.8 mg/dL vs. 1.0 mg/dL; $p = 0.031$). Among patients of *E. coli*, *K. pneumonia*, and *P. aeruginosa* BSI, 82% had systemic complications, including septic shock (75% vs. 52.9% vs. 70%), liver function impairment (53.5% vs. 29.4% vs. 50%), disseminated intravascular coagulation (46.2% vs. 35.3% vs. 30%), and acute renal function impairment (14.3% vs. 17.6% vs. 20%). The highest mortality rate was patients with *P. aeruginosa* BSI (40%) as compared with patients of *E. coli* and *K. pneumoniae* BSI (14.3% and 11.8%; Table 3).

Antibiotics susceptibility test

Thirty-two percent (9/28) of the *E. coli* isolates were resistant to cephalosporins, including 32.1% isolates (9/28)

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Table 1 Microbiological characteristics of bloodstream infections in acute leukemia children.

	ALL $n = 103$	AML $n = 23$	Total $n = 126$	p
Gram positive bacteria	42/103 (40.8)	10/23 (43.5)	52/126 (42.3)	0.879
CoNS	23/103 (22.3)	7/23 (30.4)	30/126 (23.8)	0.706
Streptococcus spp.	7/103 (6.8)	2/23 (8.7)	9/126 (7.1)	0.762
Enterococcus	2/103 (1.9)	1/23 (4.3)	3/126 (2.4)	0.507
ORSA	4/103 (3.9)	0/23 (0)	4/126 (3.2)	0.782
Others	6/103 (0.9)	0/23 (0)	6/126 (4.8)	0.547
Gram negative bacteria	59/103 (57.3)	12/23 (52.2)	71/126 (56.3)	0.965
E. coli	24/103 (23.3)	4/23 (17.4)	28/126 (22.2)	0.822
K. pneumoniae	13/103 (12.6)	4/23 (17.4)	17/126 (13.5)	0.848
P. aeruginosa	8/103 (7.8)	2/23 (8.7)	10/126 (7.9)	0.891
Enterobacter spp.	5/103 (4.9)	0/23 (0)	5/126 (4)	0.650
Others	9/103 (8.7)	2/23 (8.7)	11/126 (8.7)	0.892
Fungi[a]	2/103 (1.9)	1/23 (4.3)	3/126 (2.4)	0.507

[a] In ALL: one was *Cryptococcus neoformans*, another was *Candida albican*. In AML: *Candida tropicalis*.
Data are presented as *n*/*N* (%).
ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; CoNS = coagulase-negative staphylococci; ORSA = oxacillin-resistant *Staphylococcus aureus*; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*; *P. aeruginosa* = *Pseudomonas aeruginosa*.

resistant to first generation cephalosporin (cefazolin), 28.6% (8/28) resistant to second generation cephalosporin (cefuroxime), 25% (7/28) resistant to third generation cephalosporin (cefotaxime/ceftriaxone), six of 28 (21.4%) resistant to fourth generation cephalosporin (cefepime), and 25% (7/28) with ESBLs. Moreover, 35% (6/17) of the *K. pneumoniae* isolates were resistant to cephalosporins, 17.6% (3/17) carried with ESBLs. All the *E. coli* and *K. pneumoniae* isolates were susceptible to carbapenems. Among *P. aeruginosa* isolates, one isolate was extensively drug-resistant (10%), one isolate was MDR (10%) but there was no pan drug-resistant isolate. All *P. aeruginosa* isolates were susceptible to carbapenems and fluoroquinolones (Table 4).

Discussion

In pediatric acute leukemia patients, BSIs are the one of the most important infection complications and could lead to high mortality during the course of treatment. Approximately 3250 new cases of leukemia are diagnosed annually in the United States. ALL accounts for ~80% of cases and

Table 2 Laboratory data, systemic complications, and mortality among bloodstream infections of gram positive and gram negative bacteria in acute leukemia children.

	BSIs		Total $n = 123$	p
	GPC $n = 52$	GNB $n = 71$		
Laboratory data				
WBC $\times 10^3$ (/μL)	12.9 ± 55.4	3.3 ± 10.8	7.5 ± 35.8	0.001
ANC (/μL)	1256 ± 2135	606 ± 2037	911 ± 2256	0.041
Neutropenic status	41 (78.8)	65 (91.5)	106 (86.2)	0.038
ANC < 500 (/μL)	32 (61.5)	63 (88.7)	85 (69.1)	0.002
500 < ANC < 1000 (/μL)	4 (7.7)	2 (2.8)	6 (4.9)	0.402
1000 < ANC < 1500 (/μL)	5 (9.6)	0 (0)	5 (4.1)	0.015
Peak CRP (mg/L)	127.2 ± 138.7	179.3 ± 132.4	152.4 ± 135.3	0.005
Hb (g/dL)	10.7 ± 3.5	12.1 ± 2.3	11.1 ± 2.9	0.457
PLT $\times 10^3$ (/μL)	120 ± 142	65 ± 71	95 ± 116	0.039
Sepsis related organ failure/organ dysfunction				
Shock/hypotension	5 (9.6)	23 (32.4)	28 (22.8)	0.001
Septic embolism	3 (5.8)	10 (14.1)	13 (10.6)	0.120
Meningitis	1 (1.9)	2 (2.8)	3 (2.4)	0.653
Mortality	4 (7.8)	8 (11.1)	12 (9.8)	0.449

Data are presented as *n* (%) or mean ± standard deviation.
ANC = absolute neutrophil counts; BSIs = bloodstream infections; CRP = C-reactive protein; GPC = gram-positive cocci; GNB = Gram-negative bacteria; Hb = hemoglobin; PLT = platelet counts; WBC = white blood cell counts.

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Table 3 Laboratory data, systemic complications, and mortality among bloodstream infections of *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* in acute leukemia children.

	E. coli $n = 28$	*K. pneumoniae* $n = 17$	*P. aeruginosa* $n = 10$	Total $n = 55$	*p*
WBC $\times 10^3$ (/μL)	1.7 ± 3.2	0.6 ± 1.2	0.7 ± 0.9	1.2 ± 2.5	0.478
ANC (/μL)	713.2 ± 2518	343.2 ± 1283.9	255 ± 616	525.6 ± 1972.9	0.089
Hb (g/dL)	9.6 ± 1.5	9.4 ± 1.3	9.2 ± 2.5	9.5 ± 1.7	0.79
PLT $\times 10^3$ (/μL)	75.5 ± 87.3	41.9 ± 38.2	48.6 ± 50.8	61.5 ± 71.7	0.494
ESR (mm/h)	101.2 ± 31.3	115.3 ± 35.5	98.5 ± 30.1	102.2 ± 32.6	0.518
Dimer (ng/mL)	1192.5 ± 1119.6	1770.1 ± 1248.1	958.3 ± 158.8	1301.8 ± 1067.7	0.405
Peak CRP (mg/L)	218.6 ± 150.6	208.5 ± 106.2	135.4 ± 94.2	200.7 ± 132.6	0.312
AST (U/L)	98.8 ± 91.3	47 ± 41.7	72.4 ± 53.9	80.5 ± 77.3	0.109
ALT (U/L)	125.4 ± 111.5	86.9 ± 114.8	120.2 ± 165	114.5 ± 122.2	0.301
BUN (mg/dL)	15.4 ± 10.2	15.9 ± 7.9	15.5 ± 7.8	15.6 ± 9.1	0.734
Cr (mg/dL)	0.9 ± 1.9	1.1 ± 2.6	0.6 ± 0.4	0.9 ± 1.9	0.002
T-bil (mg/dL)	5.1 ± 5.7	2.7 ± 4.2	9.1 ± 6.7	4.8 ± 5.5	0.135
D-bil (mg/dL)	3.2 ± 4.9	1 ± 1.9	7.5 ± 6.2	3 ± 4.6	0.031
Neutropenic status	25/28 (89.3)	16/17 (94.1)	9/10 (90)	50/55 (90.9)	0.683
ANC <500 (/μL)	24/28 (85.7)	16/17 (94.1)	8/10 (80)	48/55 (87.3)	0.540
500 < ANC < 1000 (/μL)	1/28 (3.6)	0/17 (0)	0/10 (0)	1/55 (1.8)	0.617
1000 < ANC < 1500 (/μL)	0/28 (0)	0/17 (0)	1/10 (10)	1/55 (1.8)	0.105
Sepsis related organ failure/organ dysfunction					
Shock/hypotension	21/28 (75)	9/17 (52.9)	7/10 (70)	37/55 (67.3)	0.311
Liver function impairment	15/28 (53.5)	5/17 (29.4)	5/10 (50)	25/55 (45.5)	0.355
DIC	13/28 (46.2)	6/17 (35.3)	3/10 (30)	19/55 (34.5)	0.683
AKI	4/28 (14.3)	3/17 (17.6)	2/10 (20)	9/55 (16.4)	0.925
Septic embolism[a]	5/28 (17.9)	2/17 (11.8)	1/10 (10)	8/55 (14.5)	0.251
Mortality	4/28 (14.3)	2/17 (11.8)	4/10 (40)	10/55 (18.2)	0.143

[a] Septic embolism including soft tissue necrotizing fasciitis and perianal abscess.
Data are presented as *n*/*N* (%) or mean ± standard deviation.
AKI = acute kidney injury; ALT = and alanine transaminase; ANC = absolute neutrophil counts; AST = aspartate transaminase; BUN = blood urea nitrogen; Cr = creatinine; CRP = C-reactive protein; D-bil = direct bilirubin; DIC = disseminated intravascular coagulation; *E. coli* = *Escherichia coli*; ESR = erythrocyte sedimentation rate; Hb = hemoglobin; *K. pneumoniae* = *Klebsiella pneumoniae*; *P. aeruginosa* = *Pseudomonas aeruginosa*; PLT = platelet counts; T-bil = total bilirubin; WBC = white blood cell counts.

AML for the rest of 20%.[6] Among the therapeutic regimens of acute leukemia, AML patients received more cytotoxic and intense chemotherapy than ALL patients.[7] The chemotherapy protocols of Taiwan Pediatric Oncology Group for AML included cytarabine, daunomycin, etoposide, idarubicin, and 6-thioguanine. The regimens caused long duration of profound neutropenia and resulted in severe mucositis to increase the susceptibility of infections.

Table 4 Antibiotics susceptibility of *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* isolates from bloodstream infections in pediatric acute leukemia patients.

	E. coli $n = 28$	*K. pneumoniae* $n = 17$	*P. aeruginosa* $n = 10$ (%)	Total $n = 55$
1st generation cephalosporin resistance (cefazolin)	9 (32.1)	6 (35.9)	10 (100)	25 (45.5)
2nd generation cephalosporin resistance (cefuroxime)	8 (28.6)	6 (35.9)	10 (100)	23 (41.8)
3rd generation cephalosporin resistance (cefotaxime/ceftriaxone)	7 (25)	4 (23.5)	2 (20)	13 (23.6)
4th generation cephalosporin resistance (cefepime)	6 (21.4)	3 (17.6)	0 (0)	9 (16.4)
Cephalosporinases (AmpCs)	9 (32.1)	6 (35.9)	10 (100)	25 (45.5)
ESBLs	7 (25)	3 (17.6)	NA	10 (18.2)
Carbapenemase	0 (0)	0 (0)	0 (0)	0 (0)

Data are presented as *n* (%).
AmpCs = AmpC β-lactamases; *E. coli* = *Escherichia coli*; ESBLs = extended-spectrum β-lactamases; *K. pneumoniae* = *Klebsiella pneumoniae*; NA = not available; *P. aeruginosa* = *Pseudomonas aeruginosa*.

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Host factors play a part in the clinical presentations, laboratory data, and outcome of BSIs in ALL and AML patients. AML patients had a high rate of BSIs than ALL patients (79.3% vs. 61.8%). Furthermore, plasma level of CRP was significant high in AML patients and WBC significantly low in ALL patients. However, the other clinical symptoms/signs, laboratory data, and systemic complications showed no significant difference between groups.

Neutropenia was usually associated with life-threatening opportunistic infections in the setting of cytotoxic chemotherapy. These episodes of febrile neutropenia might be the result of BSIs with pathogenic micro-organisms. The duration and degrees of the neutropenia underscored the increased risk for life-threatening infections. Fever and neutropenia in hematologic malignancies patients during chemotherapy, and there were approximately 10—30% patients reported to be complicated with sepsis.[8] However, there have been limited data concerning morbidity and mortality after developing sepsis in children and adolescents with leukemia. In this study, 81.7% verified BSIs episodes occurred in association with neutropenia.

The spectrum of organisms responsible for infectious complications in leukemic hosts is daunting, since virtually any organism can become invasive if host defenses are severely impaired. In the 1980s a rise in gram-positive infections particularly due to CoNs led to a change in the spectrum of pathogens. More recently the pendulum has swung back with the re-emergence of gram-negative organisms producing ESBLs and carbapenemases. In this study, GNB (55%) were the leading pathogens of BSIs. Bacteremia due to GNB was a significant issue in both hospitalized and community-dwelling patients.[9] In a study, 45% of community-onset BSIs was due to GNB in contrast to 31% of hospital-onset infections.[10] In the United States, the National Nosocomial Infections Surveillance System reported that the proportion of GNB BSIs in intensive care unit (ICU) patients remained 25—30% from 1986 to 2003.[11] The leading pathogens of community acquired GNB BSIs were *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.[12,13] Another study also showed the distribution of community-onset GNB BSIs was: *E. coli* (76%), *P. aeruginosa* (7.9%), and *K. pneumoniae* (5.4%).[14] BSIs of *E. coli* predominated in cases of community-onset GNB. With hospital-onset GNB BSIs, the distribution of pathogens were: *E. coli* (18%), *K. pneumoniae* (16%), and *P. aeruginosa* (8%).[15] Another study of hospital-onset bacteremia in ICUs, was led by *P. aeruginosa* (22.2%), *Enterobacter* spp (22.2%), *K. pneumoniae* (17.8%), *E. coli* (15.6%), and *Serratia marcescens* (11.1%).[16] The percentage of *P. aeruginosa* BSIs was frequently high among ICU patients. Patients in ICU more frequent received antibiotics, which increased the risk of infections with *P. aeruginosa*.

GNB sepsis with shock has a mortality rate of 12—38%; mortality varies depending, in part, on whether the patient received timely and appropriate antibiotic therapy.[17—19] But those organisms had serious therapeutic problems because of the increasing incidence of MDR.[9] MDR bacteria accounted for 44% of the identified bacteria in a previous study.[20] The burden of antimicrobial resistance among GNB BSIs was substantial. Since the 1990s, the rise of MDR GNB infections had posed a major clinical problem worldwide. MDR GNB infections result in increased morbidity and mortality and have now made their way to children.[21] The most common resistant GNB to antibiotics including three important β-lactamases: cephalosporinases of AmpC β-lactamases, ESBLs, and carbapenemases.[22]

MDR *P. aeruginosa* was found with high mortality than susceptible *P. aeruginosa* in patients (36% vs. 27%).[23] However, MDR *P. aeruginosa* was not a risk factor associated to mortality by multivariate analysis. Among those BSIs of MDR *P. aeruginosa*, the mortality of used appropriate antibiotics and with no antibiotic used was 83.3% and 18.8% ($p = 0.011$).[24] In addition, another retrospective study of BSIs of MDR GNB demonstrated that MDR GNB was not associated with increased mortality.[23] Whereas, it was associated with the length of hospitalization but not the susceptibility of pathogens.[24] In our study, MDR GNB account for 45.5% isolates among *E. coli*, *K. pneumonia*, and *P. aeruginosa*. However, these isolates were not associated with significant high mortality. BSIs of MDR GNB led to high morbidity and mortality may be associated with inappropriate initial antibiotic treatment.

This study had several limitations. Firstly, the clinical presentations, physical examinations, and laboratory data were obtained from medical records, so some incomplete data was inevitable due to the retrospective design. Secondly, the case numbers were small, some characteristics of clinical and laboratory items showed only marginal on limited statistical significance.

In conclusion, BSIs of pediatric acute leukemic patients remain severe infections and complications. The risk factors associated with BSIs were GNB pathogens and neutropenic fever. However, the case-fatality rate was still high under broad spectrum antimicrobial therapy. Early identification of BSIs and implementation of empiric antibiotics is essential in managing leukemic patients with neutropenic fever.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Cheng J, Sakamoto KM. Topics in pediatric leukemia — acute myeloid leukemia. *Med Gen Med* 2005;**7**:20.
2. Smiley S, Almyroudis N, Segal BH. Epidemiology and management of opportunistic infections in immunocompromised patients with cancer. *Abstr Hematol Oncol* 2005;**8**:20—30.
3. Fisher RI, DeVita Jr VT, Bostick F, Vanhaelen C, Howser DM, Hubbard SM, et al. Persistent immunologic abnormalities in long-term survivors of advanced Hodgkin's disease. *Ann Intern Med* 1980;**92**:595—9.
4. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility test: Approved standard M2—A7*. 7th ed. PA: Clinical and Laboratory Standards Institute; 2000. 2000.
5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;**18**:268—81.
6. O'Brien M, Lacayo N. Acute leukemia in children. *Dis Mon* 2008;**54**:202—25.

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7. Kaspers GJL, Creutzig U. Pediatric acute myeloid leukemia: international progress and future directions. *Leukemia* 2005; **19**:2025—9.
8. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**524**:e56—93.
9. Suárez CJ, Lolans K, Villegas MV, Quinn JP. Mechanisms of resistance to beta-lactams in some common Gram-negative bacteria causing nosocomial infections. *Expert Rev Anti Infect Ther* 2005;**3**:915—22.
10. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. *J Clin Microbiol* 2003; **41**:3655—60.
11. Gaynes R, Edwards JR. National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis* 2005;**41**:848—54.
12. Love LJ, Schimpff SC, Schiffer CA, Wiernik PH. Improved prognosis for granulocytopenic patients with gram-negative bacteremia. *Am J Med* 1980;**68**:643—8.
13. Pizzo PA, Hathorn JW, Hiemenz JW, Browne M, Commers J, Cotton D, et al. A randomized trial comparing ceftazidime alone with combination antibiotic therapy in cancer patients with fever and neutropenia. *N Engl J Med* 1986;**315**: 552—8.
14. Luzzaro F, Viganò EF, Fossati D, Grossi A, Sala A, Sturla C, et al. Prevalence and drug susceptibility of pathogens causing bloodstream infections in northern Italy: a two-year study in 16 hospitals. *Eur J Clin Microbiol Infect Dis* 2002;**21**:849—55.
15. Shorr AF, Tabak YP, Killian AD, Gupta V, Liu LZ, Kollef MH. Healthcare-associated bloodstream infection: A distinct entity? Insights from a large U.S. database. *Crit Care Med* 2006; **34**:2588—95.
16. Sligl W, Taylor G, Brindley PG. Five years of nosocomial gram-negative bacteremia in a general intensive care unit: epidemiology, antimicrobial susceptibility patterns, and outcomes. *Int J Infect Dis* 2006;**10**:320—5.
17. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. *Antimicrob Agents Chemother* 2005;**49**:760—6.
18. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006;**34**:1589—96.
19. Gikas A, Samonis G, Christidou A, Papadakis J, Kofteridis D, Tselentis Y, et al. Gram-negative bacteremia in non-neutropenic patients: a 3-year review. *Infection* 1998;**26**:155—9.
20. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009;**302**:2323—9.
21. Logan LK. Carbapenem-resistant *Enterobacteriaceae*: an emerging problem in children. *Clin Infect Dis* 2012;**55**:852—9.
22. Hsu AJ, Tamma PD. Treatment of multidrug-resistant gram-negative infections in children. *Clin Infect Dis* 2014;**58**: 1439—48.
23. Cattaneo C, Antoniazzi F, Casari S, Ravizzola G, Gelmi M, Pagani C, et al. *P. aeruginosa* bloodstream infections among hematological patients: an old or new question? *Ann Hematol* 2012;**91**:1299—304.
24. Lye DC, Earnest A, Ling ML, Lee TE, Yong HC, Fisher DA, et al. The impact of multidrug resistance in healthcare-associated and nosocomial gram-negative bacteraemia on mortality and length of stay: cohort study. *Clin Microbiol Infect* 2012;**18**: 502—8.

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