

Original Article

Antimicrobial Susceptibility Pattern of Bacteria Isolated from Blood Urine and Wound Swab in Febrile Neutropenic Children in a Tertiary Level Hospital, Bangladesh.

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Abstract

Fever is the commonest symptom of infection in neutropenic children with malignancy, under chemotherapy. To avoid bacterial complications, it is necessary to diagnose infection early and to administer empirical antibiotic. The aim of this study was to find out the causative bacteria and their antimicrobial susceptibility pattern in febrile neutropenic children who were under chemotherapy. The study was conducted in a tertiary level hospital, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Blood, urine and wound swab were collected for culture from 30 febrile neutropenic children, age 1-15 years. Out of 70 samples total 26(37.14%) yielded growth of bacteria among which 6(20%), 12(40%), and 8 (80%) were isolated from blood, urine and wound swab respectively. Among the bacteria *Pseudomonas* spp, *Esche coli* and *Staph aureus* were most frequently isolated. Meropenem, imipenem and netilmicin were the most sensitive against gram negative bacteria and the entire isolated gram positive bacteria were 100% sensitive to vancomycin and linezolid.

Key words: Antimicrobial susceptibility, bacteria, blood, neutropenic children.

Introduction

Cancer patients, especially those with haematological and lymphoid malignancies, in whom infectious complications represent an important cause of morbidity and mortality, as chemotherapy results in neutropenia in a vast majority of patients.¹ Bacterial infections are common and potentially serious complications of cancer treatment.² However, this has been obtained with aggressive use of chemotherapy and more use of invasive procedures, which may also contribute to increased susceptibility to infections.³ In addition, disruptions of the physical defense barriers of skin and mucosa, secondary to chemotherapy, leads to increased exposure to potentially pathogenic micro-organisms. In situ venous access catheters are further risk factors for bacteremia.²

Over the past decade there has been a considerable change in the pattern of pathogens causing infections in cancer patients.¹ A low nadir in the neutrophil count

and protracted neutropenia (i.e. neutrophil count of <500 cells/cmm for 10 days) are major risk factors for impending infection. In addition to quantitative changes in neutrophil counts, abnormalities of phagocytic function or other deficits in the immune response may further increase the risk for infection in a neutropenic host.⁴ Among the neutropenic children with febrile, *Staphylococci* (both coagulase-negative and coagulase-positive) and *Escherichia coli* are the most frequently isolated gram-positive and gram-negative organisms respectively.^{5,6}

Beta-lactam and/or aminoglycoside combination for febrile neutropenia is considered better approach, because of its broader spectrum, potential synergistic activity against gram-negative rods, and its potential ability to reduce the emergence of resistant strains.^{4,6} With time the resistance to beta-lactam antibiotics has been on the rise and warrants close monitoring of the sensitivity patterns of different organism regularly.⁵

Though the exact statistics of incidence and prevalence of febrile neutropenia among the patients with cancer in our country is not available but it is the common opinion that the number of such kind of problem is increasing day by day. The aim of this study was to determine the microbiological spectrum by culture of different clinical

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samples during episodes of fever and neutropenia in children with cancer.

Methodology and Method

This prospective cross sectional study was conducted in the Department of Paediatric Haematology and Oncology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh for a period of 6 months (March-September, 2010). All microbiological works were done in the Department of Microbiology and Immunology, BSMMU.

Study population: Thirty children between the age of 1 to 15 years with marrow/biopsy evidence of malignancy and undergoing chemotherapy with clinical features of febrile neutropenia in the Department of Paediatric Haematology and Oncology, BSMMU were included.

Data collection tool: Structured questionnaire and a check list.

Data collection technique: The diagnosis of febrile neutropenia (FN) was made as absolute neutrophil count (ANC) ≤ 500 cells/cmm with single oral temperature of $\geq 38.3^\circ\text{C}$ or a temperature of $\geq 38.0^\circ\text{C}$ for ≥ 1 hour.⁷ All participants were subjected to be thorough clinical examination and tests for hemoglobin, complete blood cell count, peripheral blood film.

Sample collection for culture: Blood, urine, and wound swab were collected for culture under all aseptic precautions and according to standard WHO guideline.⁸ Trypticase soy broth was used for primary culture of blood. Blood agar, MacConkey agar and Chocolate agar media were used for subculture of blood. Urine and wound swab were inoculated in blood agar and MacConkey agar media. All inoculated media were incubated at 37°C aerobically over night. In culture positive cases, bacterial identification was done by colony morphology, gram staining and different biochemical tests as per standard guide line.⁹

Antimicrobial susceptibility testing: Antibiotic susceptibility pattern were determined by Kirby-Bauer disc diffusion method¹⁰ using commercially available antibiotics discs (Oxoid, UK). The organisms were tested against routinely used antibiotics like amikacin, gentamycin, imipenem, meropenem, ceftazidime, ceftriaxone, co-trimoxazole, netilmicin. Zone of inhibition was recorded as sensitive or resistant according to CLSI guideline.¹¹

Result:

Out of total 70 samples 26(37.1%) yielded growth of bacteria and among them 6(20%) were blood, 12(40%) were urine and 8(80%) were wound swab (Table-I).

Of the six bacteria isolated from blood, 2(33.3%) each were *Pseudomonas* spp and *Staph aureus*. Among the 12 bacteria isolated from urine samples *Esch. coli* were 6 (50%) and *Pseudomonas* spp. were the maximum number 4 (50%) isolated from wound swab culture (Table-II).

Among the 26 culture positive cases 10 (38.4%) had ANC count more than 100 cells/cmm and 16 (61.6%) had ANC count less than 100 cells/cmm (Table-III).

Most of the gram negative bacteria were sensitive to meropenem, imipenem and netilmicin and *Staph aureus*, β hemolytic *Streptococci* and *Enterococcus* spp were 100% sensitive to vancomycin and linezolid (Table-IV).

Table-I: Distribution of blood, urine & wound swab culture of the patients (N=70)

Specimen	Positive n (%)	Negative n (%)
Blood(N=30)	6 (20)	24 (80)
Urine(N=30)	12 (40)	18 (60)
Wound swab(N=10)	8 (80)	2 (20)
Total (N=70)	26 (37.1)	54 (62.9)

N=Total number of samples. n= positive samples

Table-II: Distribution of organisms isolated from blood, urine and wound swab.

Organisms	blood n (%)	urine n (%)	wound swab n (%)	total n (%)
<i>Pseudomonas</i> spp	2 (33.3)	2 (16.7)	4 (50)	8 (16.7)
<i>Esch. coli</i>	1 (16.7)	6 (50)	0 (0)	7 (16.7)
<i>Klebsiella</i> spp	0 (0)	2 (16.7)	0 (0)	2 (16.7)
<i>Acinetobacter</i> spp	1 (16.7)	0 (0)	0 (0)	1 (16.7)
<i>Staph aureus</i>	2 (33.3)	0 (0)	3 (37.5)	5 (16.7)
<i>Enterococcus</i> spp	0 (0)	2 (16.7)	0 (0)	2 (16.7)
β hemolytic <i>Streptococci</i>	0 (0)	0 (0)	1 (12.5)	1 (0)
Total	6 (100)	12 (100)	8 (100)	26 (100)

Table-III: Absolute neutrophil count (ANC) among culture positive patients (n=26)

ANC	Number (%)
>100 cells/cmm	10 (38.4)
<100 cells/cmm	16 (61.6)
Total	26 (100)

Table-IV: Antimicrobial sensitivity pattern of isolated bacteria from blood, urine and wound swab.

Isolated organism	Sensitive (%)										
	Ak	Cro	Caz	G	Cip	Mem	Ipm	Net	Cot	Va	Lzd
<i>Pseudomonas</i>	75	25	75	50	50	87.5	100	87.5	25	-	-
<i>Esch coli</i>	71.4	71.4	57.1	71.4	42.9	100	100	71.4	57.1	-	-
<i>Klebsiella</i> spp	50	100	0	50	0	100	100	100	50	-	-
<i>Acinetobacter</i>	100	0	0	0	0	0	100	100	-	-	-
<i>Staph aureus</i>	80	60	60	60	40	-	-	-	80	100	100
<i>Enterococcus</i> spp	50	-	-	0	100	100	100	-	50	100	100
β hemolytic <i>Streptococci</i>	100	100	0	100	0	-	-	-	100	100	100

AK-amikacin, Cro-ceftriaxone, Caz-ceftazidime, G-gentamicin, Cip-ciprofloxacin, Mem-meropenem, Ipm-imipenem, Net-netilmicin, Cot-cotrimoxazole, Va-vancomycin, Lzd-linezolid.

Discussion:

Febrile neutropenia (FN) is a frequent and potentially life-threatening complication in children with cancer under chemotherapy.⁷ In addition to quantitative changes in neutrophil counts, abnormalities of phagocytic function or other deficits in the immune response may further increase the risk for infection in a neutropenic host.⁴ Fever during chemotherapy-induced neutropenia can be the first sign of bacterial infection and therefore, requires prompt and careful attention. Children with cancer can be severely immune compromised and most chemotherapeutic agents also inhibit the inflammatory response to invading microbes.¹² Bacterial infection is common and potentially serious complication of cancer treatment. The etiology, clinical course, and outcome of fever and neutropenia in children with cancer using the current guidelines and diagnostic resources should be regularly upgraded.

A total of 70 samples from 30 children with marrow/biopsy evidence of malignancy and clinical features of febrile neutropenia were included. Out of 26 culture positive cases 6 (23.2%), 12(46.1%) and 8 (30.7%) bacteria were isolated from blood, urine and wound swab culture respectively. Isais-Agdeppa and Bravo in a retrospective study determined the bacterial blood isolates of pediatric cancer.⁷ A total of 90 patients were included and 7% of the patients had growth in the blood culture. In this study bloodstream infection is higher (23.2%) than the previous study.⁷ It is probably due to higher number children had absolute neutrophil count (ANC) <100. Patients with neutrophil count of <100 cells/cmm are at greater risk than are those with counts of <500 cells/cmm.⁴ Jones et al reported that blood cultures were positive in 58% cases of fever episodes with an absolute neutrophil count <500/cmm.¹³ Bacterial infection rate increased when an absolute neutrophil count <500/cmm were also observed in other studies in different geographical areas.^{14,15}

In this study distribution among 26 patients with culture positive cases 10 (30.3%) had ANC count more than 100 cells/cmm and 23 (69.7%) had ANC count less than 100 cells/cmm. So bacterial infection rate was significantly higher in those cases had ANC count less than 100 cells/cmm and these findings were similar with the previous study. Hana *et al* studied the etiology, clinical course, and outcome of fever and neutropenia in

children with cancer.⁶ Infection was proven in 25% and bacteremia accounted for 41% of the proven bacterial episodes, with *Viridans streptococci* 13%, *Pseudomonas* spp. 6% and *Escherichia coli* 6% were the most frequently isolated organisms. Mahmud et al observed total 29 bacteria were cultured in 62 febrile episodes.¹⁶ Among them 55% organisms were isolated from blood and 45% from other sites with 51.7% and 48.3% were gram negative and positive bacteria respectively.

In this study Isolation from blood was 23.3% and from other sites 76.8% and the isolation rate of gram negative bacteria was higher than the gram positive bacteria. Isolated gram positive bacteria were 100% sensitive to vancomycin and linezolid whereas gram negative bacteria were most sensitive to meropenem, imipenem and netilmicin in this study. Antibiotic prophylaxis reduces the incidence of fever, but its routine use remains controversial for patients at low risk of neutropenic infection.^{17,18} The most serious form of sepsis originates from the gram-negative group and empiric antibiotic regimens offer good control over this group.¹⁹ A typical choice would be an anti-pseudomonal beta-lactam and an aminoglycoside, e.g. piperacillin/tazobactam plus amikacin.²⁰ Other monotherapy regimens of 3rd or 4th generation cephalosporins with anti-pseudomonal activity, like ceftazidime or cefepime, may also be used provided cognizance, pleading.²¹

Despite improvement in supportive care measures, most febrile, neutropenic patients need close observation and empiric intravenous antibiotic therapy. So, culture and susceptibility test should be performed regularly for proper treatment and management of febrile neutropenic children under chemotherapy.

Ethical consideration

Prior to commencement of this study, the research protocol was approved by ethical committee of Bangladesh College of Physician and Surgeons, Dhaka. Informed consent was obtained from the legal guardian of the children.

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Conflict of interest

We do not have any potential conflicts of interest.

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