

Bacteriological Profile of Bloodstream Infections in Paediatric Oncology Patients with Febrile Neutropenia: A Five-year Ambispective Study at a Tertiary Care Centre in Northern India

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ABSTRACT

Introduction: Bloodstream Infections (BSI) are known to be responsible for significant mortality and morbidity. Cancer patients, being immunosuppressed, are more vulnerable to developing infections and often present with Febrile Neutropenia (FN).

Aim: To determine the epidemiology, microbiology, and antibiotic susceptibility pattern among paediatric cancer patients admitted with FN.

Materials and Methods: This ambispective study was conducted over a period of 5 years, from April 2018 to April 2023, in the Department of Paediatric Oncology in coordination with the Department of Microbiology at Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India. Isolates collected from April 2018 to April 2022 were retrospectively evaluated with respect to gender, age, and severity of neutropenia, whereas those analysed prospectively were from May 2022 to April 2023. Data from 328 patients presenting with 420 episodes of FN were obtained, including information on age, gender, the organism isolated, and antibiotic susceptibility pattern. Blood samples were collected in blood culture bottles and incubated at 35±2°C in the automated BACT/ALERT blood culture system. Positive bottles were further subjected to manual identification and antibiotic susceptibility testing of the bacterial isolates using the Kirby Bauer disk diffusion method.

The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines of 2023. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software v.23.

Results: A total of 328 patients were admitted over the five-year period, with 420 episodes of FN. The culture positivity rate was 81 out of 420 (19.2%). Among the patients, 50 (61.7%) were males, and 36 (45%) had moderate neutropenia at the time of admission. The most common malignancy was Acute Lymphoblastic Leukaemia (ALL), accounting for 25 cases (30.8%), followed by neuroblastoma with 13 cases (16%) and retinoblastoma with 1 case (1.2%). Coagulase-negative *Staphylococci* (CoNS) were isolated in 21 cases (25.9%). Furthermore, 12 (75%) of the *Staphylococcus* isolates and 12 (57.14%) of the CoNS were found to be methicillin-resistant. Among the gram-negative bacteria, *Acinetobacter baumannii* showed the highest resistance to the tested antibiotics.

Conclusion: The majority of cases were moderately neutropenic, and CoNS was found to be the most common pathogen in BSI. None of the isolates were pan drug-resistant. Empiric antibiotic treatment for FN should be tailored to the locally prevalent pathogens and their susceptibility patterns. This approach will aid in appropriate infection control practices, ultimately reducing mortality and morbidity in affected patients.

Keywords: Antimicrobial susceptibility, Paediatric cancers, Retinoblastoma

INTRODUCTION

Multimodal chemotherapy used to treat malignant diseases may cause serious infections in children, which have a negative effect on the quality of life during treatment [1,2]. The underlying disease, therapies used for treatment, and the technology and medical tools employed facilitate infections in this subset of patients caused by microorganisms that do not otherwise exhibit pathogenicity [3]. One of the most common complications associated with the therapy is FN, which is defined as a single spike in oral temperature rising to or greater than 101°F, or a temperature greater than or equal to 100.4°F (38°C) for at least one hour, with an Absolute Neutrophilic Count (ANC) of less than 1500 cells/microlitre [4]. The low neutrophil counts account for significant morbidity and mortality in these patients [5]. Infections are managed by using appropriate empirical antimicrobial therapy based on a comprehensive understanding of the commonly encountered pathogens and antibiotic sensitivity patterns [6].

Over the last 40 years, the spectrum of microorganisms isolated from febrile neutropenic patients has undergone a major change. Until the mid-1980s, Gram-negative bacilli such as *Escherichia coli*,

Klebsiella spp., and *Pseudomonas aeruginosa*, as well as Gram-positive cocci like *Staphylococcus aureus*, were most frequently isolated from this group of patients. The spectrum has now shifted towards CoNS and the viridans group of *streptococci* [7].

The pathogens responsible for causing infections and their resistance patterns are determined by certain factors, such as differences in chemotherapy and prophylaxis regimens, the use of central venous catheters, hospital environment, and the climate of the region. These factors should be considered as they play a role in the selection of appropriate initial antibiotics [2].

The most common infections in cancer patients include BSIs, Urinary Tract Infections (UTIs), and bacterial pneumonias [8-10]. A comparison of mortality rates related to infections reveals higher rates in developing countries than in developed ones, and the identification and treatment of causative agents can significantly improve survival rates among paediatric cancer patients [11].

The microbiological pattern of organisms responsible for causing infections in paediatric oncology patients varies from one geographical location to another. This highlights the importance

of information on bacterial patterns and antibiotic susceptibility in modulating antimicrobial policies, which, in turn, can help reduce mortality and morbidity related to infections. The aim of the present study was to determine the epidemiology, microbiology, and antibiotic susceptibility patterns of various bacteria isolated from blood in paediatric cancer patients admitted with FN. This information will aid in increasing the efficiency of empirical antibiotic treatment regimens employed in this setting.

MATERIALS AND METHODS

This ambispective study was conducted for a period of 5 years from April 2018 to April 2023 in Department of Paediatric oncology in co-ordination with Department of Microbiology, Sher-i-Kashmir institute of medical sciences, Srinagar, Jammu and Kashmir, India. Isolates collected from April 2018 to April 2022 were evaluated retrospectively with respect to gender, age and severity of neutropenia whereas for those analysed prospectively from May 2022 to April 2023. Ethical clearance was obtained from Institutes Ethical Clearance Committee bearing number: 1556. Written informed consent was sought from the parents/caretakers of the children.

As this was a time-bound study samples available in the study duration were considered in the study.

Inclusion criteria: All paediatric cancer patients admitted to the Department of paediatric oncology with FN were included in the study.

Exclusion criteria: Patients admitted with non neutropenic fever were excluded from the study.

Study Procedure

Data of 328 patients presenting as 420 episodes of FN were obtained with respect to the age, gender, the organism isolated and the antibiotic susceptibility pattern. A neutrophil count of ANCs of $1.0-1.5 \text{ G L}^{-1}$ and $0.5-1.0 \text{ G L}^{-1}$ and $<0.5 \text{ G L}^{-1}$ was taken as mild, moderate and severe neutropenia, respectively [12]. For the samples evaluated prospectively, 5 mL of the venous blood sample was obtained aseptically from each patient and inoculated in the blood culture bottle. Inoculated blood bottles were incubated at $35 \pm 2^\circ\text{C}$ in the BacT/ALERT blood culture System and the bottles that showed a positive signal were subjected to microscopic examination of gram-stained smears of their contents. At the same time, subcultures were performed from these bottles on Blood and MacConkey agar plates, respectively, which were incubated aerobically at 37°C overnight. Any growth obtained on culture plates was studied on the following day and subjected to further identification by manual biochemical methods. Antibiotic susceptibility testing of bacterial isolates was performed using Kirby Bauer disk diffusion method, the results of which were interpreted as per Clinical and Laboratory Standards Institute guidelines 2023 [13]. American Type Culture Collection control strains, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains were used to ensure the quality of each procedure.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS software version 23.0. Categorical variables were presented as frequency and percentage, and appropriate tables were used to describe the data. Continuous variables were summarised using descriptive statistics, including the mean and standard deviation of the variables. Descriptive statistics were also used to present the summary of continuous variables.

RESULTS

A total of 420 episodes of FN were recorded in 328 patients, and blood cultures were positive in 81 (19.2%) and negative in 339 (80.7%) patients. The majority of the patients were male, accounting for 50 (61.7%) [Table/Fig-1]. The majority of the patients had moderate neutropenia at the time of admission [Table/Fig-2]. ALL accounted for 25 (30.8%) cases, making it the most common

malignancy in patients admitted with FN. This was followed by neuroblastoma with 13 cases (16%), Wilms tumour with 10 cases (12.3%), Osteosarcoma with 9 cases (11.1%), Non-Hodgkin's Lymphoma (NHL) with 9 cases (11.1%), Ewing's sarcoma with 5 cases (6.1%), Hodgkin's Lymphoma (HL) with 4 cases (4.9%), Rhabdomyosarcoma with 2 cases (2.4%), Acute Myeloid Leukaemia (AML) with 2 cases (2.4%), Meduloblastoma with 1 case (1.2%), and Retinoblastoma with 1 case (1.2%) [Table/Fig-3].

Gender	No. of cases n (%)
Male	50 (61.7%)
Female	31 (38.3%)
Total	81 (100%)

[Table/Fig-1]: Gender-wise distribution of patients included in the study.

Severity of Neutropenia	No. of cases n (%)
Mild	27 (33%)
Moderate	36 (45%)
Severe	18 (22%)
Total	81 (100%)

[Table/Fig-2]: Severity of neutropenia in the paediatric cancer patients at the time of admission.

Diagnosis	No. of cases n (%)
ALL	25 (30.8%)
Neuroblastoma	13 (16%)
Wilms tumour	10 (12.3%)
Osteosarcoma	9 (11.1%)
NHL	9 (11.1%)
Ewings Sarcoma	5 (6.1%)
HL	4 (4.9%)
Rhabdomyosarcoma	2 (2.4%)
AML	2 (2.4%)
Meduloblastoma	1 (1.2%)
Retinoblastoma	1 (1.2%)
Total	81 (100%)

[Table/Fig-3]: Distribution of paediatric patients admitted with Febrile Neutropenia (FN) with respect to clinical diagnosis.

ALL: Acute lymphoblastic leukaemia; NHL: Non-Hodgkin's lymphoma; HL: Hodgkin's lymphoma; AML: Acute myeloid leukaemia

The CoNS accounted for 21 (25.9%) cases and was the most commonly isolated bacteria, while *Enterococcus* was the least isolated with 2 cases (2.4%) [Table/Fig-4].

Bacteria isolated	n (%)
CoNS	21 (25.9)
<i>Staphylococcus aureus</i>	16 (19.7)
<i>Enterococcus</i>	2 (2.4)
<i>Escherichia coli</i>	15 (18.5)
<i>Acinetobacter baumannii</i>	11 (13.5)
<i>Klebsiella pneumoniae</i>	8 (9.8)
<i>Pseudomonas aeruginosa</i>	8 (9.8)
Total	81 (100)

[Table/Fig-4]: Frequency distribution of bacteria isolated from blood samples of febrile neutropenic paediatric patients.

CoNS: Coagulase-negative *Staphylococci*

The majority of *Staphylococcus* isolates, specifically 12 (75%), were resistant to Methicillin. Additionally, 12 (57.14%) of the CoNS isolates were methicillin-resistant [Table/Fig-5].

Acinetobacter baumannii showed the highest resistance to antibiotics, and none of the Gram-negative isolates were resistant to Tigecycline or polymyxin B [Table/Fig-6].

Organism	Number of isolates	Penicillin	Cefoxitin	Ampicillin	Vancomycin	Clindamycin	Erythromycin	Linezolid	Cotrimoxazole
<i>Staphylococcus aureus</i>	16	11 (68.7)	12 (75)	-	00	10 (62.5)	09 (56.2)	00	09 (56.2)
CoNS	21	13 (61.9)	12 (57.14)	-	00	08 (38.09)	07 (33.3)	00	07 (33.3)
<i>Enterococcus spp.</i>	2	01 (50)	00	01 (50)	00	-	-	00	00

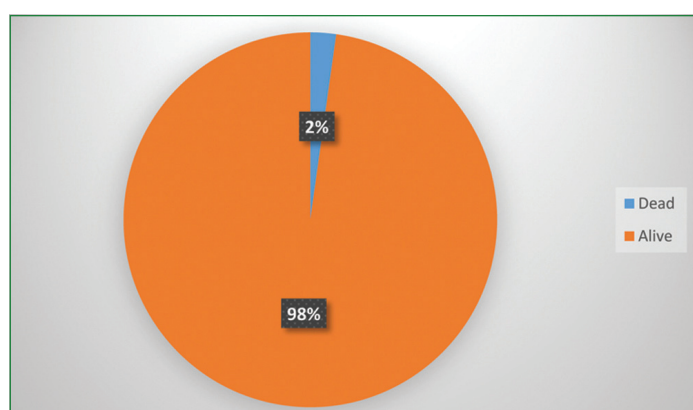
[Table/Fig-5]: Resistance pattern of the antibiotics tested against the Gram-positive isolates.

CoNS: Coagulase-negative staphylococcus

Organism	Number of isolates	Ampicillin	Ampicillin+Subactam	Amoxicillin+Clavulanate	Piperacillin+Tazobactam	Amikacin	Gen-tamycin	Cefepime	Ceftazi-dime	Ceftri-axone	Mero-penem	Imi-penem	CP	Levo	Cot
<i>Escherichia coli</i>	15	11 (73.3)	06 (40)	07 (46.6)	08 (53.3)	09 (60)	09 (60)	06 (40)	05 (33.3)	08 (53.3)	05 (33.3)	04 (26.6)	6 (40)	8 (53.3)	05 (33.3)
<i>Acinetobacter Baumannii</i>	11	-	08 (72.7)	07 (63.6)	10 (90.9)	10 (90.9)	10 (90.9)	11 (100)	11 (100)	09 (81.8)	08 (72.7)	06 (54.5)	8 (72.7)	7 (63.6)	6 (54.5)
<i>Pseudomonas aeruginosa</i>	8	-	07 (87.5)	06 (75)	05 (62.5)	05 (62.5)	06 (75)	07 (87.5)	04 (50)	-	03 (37.5)	03 (37.5)	4 (50)	2 (25)	-
<i>Klebsiella pneumoniae</i>	8	-	06 (75)	05 (62.5)	03 (37.5)	03 (37.5)	4 (50)	05 (62.5)	04 (50)	4 (50)	3 (37.5)	2 (25)	4 (50)	4 (50)	3 (37.5)

[Table/Fig-6]: Resistance pattern of the antibiotics tested against the Gram-negative isolates.

The majority of the patients (98%) were treated successfully, and the mortality rate was 2% [Table/Fig-7].



[Table/Fig-7]: Outcome of the admitted paediatric febrile neutropenic patients.

DISCUSSION

Neutropenic children are prone to infections, and the increased susceptibility to infections in this subset of patients poses a major challenge to clinicians. A decreased neutrophil count is responsible for complications, and FN is an important cause of death, especially in these patients. Appropriate use of broad-spectrum antibiotics can significantly decrease mortality in these neutropenic patients [14].

The present study was conducted at a tertiary care centre in Jammu and Kashmir. The present study population included 328 patients, showing 420 episodes of FN, and only 19.2% of the febrile episodes were microbiologically documented infections.

A total of 50 (61.7%) of the children were male. These results were consistent with the study conducted by Jacob LA et al., where he found that 51% of the children admitted as cases of FN were males [7]. Sneha L et al., and Jungrungrueng T et al., also found that 64% and 56.6% of the admitted paediatric patients were males, respectively [15,16]. Due to the association between Glutathione S-Transferase (GST) and certain cytochrome P-450 alleles that exert a protective effect on women, males have a larger prevalence than women in the haematologic malignancy group. Additionally, certain Human Leukocyte Antigen 2 DR isotypes (HLA-DR) (HLA-DRB4*01) and the C282Y mutation in the Homeostatic Iron Regulator (HFE) gene are associated with an increased risk of developing ALL in males [17].

ALL was the most common diagnosis in the present study, followed by Neuroblastoma, whereas Retinoblastoma was the least common. These results were consistent with the studies conducted by Boeriu E et al., and Dhanaleha P et al., who found ALL to be the most common diagnosis in patients admitted with FN [18,19]. However,

Lee JH et al., found in their study that AML (n=150, 44.6%) was the most common diagnosis, followed by ALL (n=112, 33.3%) [20].

It was observed that 45% of the admitted patients had moderate neutropenia, 33% had mild neutropenia, whereas 22% had severe neutropenia. Contrary to this, Jacob LA et al., found in their study that 44 (58.6%) of the episodes had profound neutropenia with ANC $\leq 100/\text{mm}^3$ [7]. Hospitalisation and timely intervention lead to the management of cases before they develop profound neutropenia.

The CoNS was found to be the most common pathogen isolated. These results were in concordance with those found by Mvalo T et al., and Celeby S et al., [21,22]. On the other hand, Gram-negative bacteria like *Escherichia coli* and *Klebsiella pneumoniae* have been reported as the most commonly involved microorganisms in cancer patients in various studies carried out in different parts of the world [7,16,19,23-26]. The higher prevalence of gram-positive cocci in the present study could be due to more use of central venous catheters, fluoroquinolone prophylaxis, aggressive antineoplastic regimes responsible for severe oropharyngeal mucositis and bowel damage, and H2 receptor blockers. A greater prevalence of Gram-negative rods as the cause of BSI among cancer patients could be due to relatively lower use of indwelling catheters and other portal devices [26].

None of the Gram-positive isolates were found to be resistant to either vancomycin or linezolid. Methicillin-resistant CoNS accounted for 57.14% of the isolates, whereas 75% of *Staphylococcus aureus* isolates were methicillin-resistant. Among the Gram-negative bacteria, *Acinetobacter baumannii* constituted the majority of multi-drug resistant isolates, with 90.9% being resistant to piperacillin-tazobactam, 72.7% being resistant to meropenem, and 54.5% being resistant to imipenem. *Klebsiella pneumoniae* was the least drug-resistant bacillus, with 75% of isolates resistant to ampicillin-sulbactam, and 37.5% and 25% of isolates being resistant to meropenem and imipenem, respectively. None of the isolates were found to be pan drug-resistant. A study carried out by Nirmal G et al., suggested otherwise, as they found that *Klebsiella* species was the most carbapenem-resistant, followed by *Escherichia coli* and the sensitivity to Tigecycline was only 33% [26]. Dharmapalan D et al., collected data from 2000 to 2015 and found that methicillin-resistant Staphylococci constituted only 50%, whereas the present study found that more than 70% of the isolates were resistant to methicillin. However, the resistance was maximum for *Klebsiella pneumoniae* only [27]. Amanati A et al., in their study found that *Acinetobacter* species, *Pseudomonas* species, *Escherichia coli*, and *K. pneumoniae* were the most common multidrug-resistant bacteria recovered from blood in oncology patients [28]. The results in the present study can be attributed to an increased prevalence of multidrug-resistant and extensively drug-resistant non fermenter isolates in this setting, thus necessitating the need to follow infection control practices and strengthen the antibiotic policy.

The mortality rate was 2%, and these results were in concordance with those found by Basu SK et al., in their study, who stated the mortality rate to be 3%. This can be attributed to the timely admission and treatment of the patients [29].

Limitation(s)

The study was conducted retrospectively for four years, which made it difficult to collect data on the chemotherapeutic protocols used and antibiotics administered prior to sample collection, if any. Therefore, this data could not be included in the study. Additionally, the data was collected from a single centre. Prospective multicentre studies need to be conducted to further validate the pattern of infection in this part of the country.

CONCLUSION(S)

Early treatment of FN patients has implications for eventual morbidity and mortality. Starting pre-emptive appropriate antibiotics is based on the local antibiotic susceptibility profiles at different centres. The present study clearly shows that mortality is significantly reduced by early institution and appropriate antibiotic usage. Framing an antibiotic policy, especially in a subset of patients who are prone to infections is very critical. Gaining insight into the microbiological profile of the microbiologically documented infections plays an important role in enhancing antibiotic stewardship and infection prevention and control measures. The high prevalence of Gram-positive cocci as the cause of FN emphasises the need for antiseptic precautions to be taken while using intravenous catheters. The absence of resistance to the last resort antibiotics implies that a strict antibiotic policy has to be followed for it to be maintained.

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