DOI: 10.7860/JCDR/2021/49887.15748

Original Article

Microbiology Section

A Study on Clinicomicrobiological Profile of Infections in Febrile Neutropenic Children with Haematological Malignancies in a Tertiary Care Hospital at Chennai, Tamil Nadu, India

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ABSTRACT

Introduction: Febrile neutropenia is common with chemotherapy regimens in 25-40% of patient, especially in children and the severity which depends on the chemotherapy regimens. Neutropenic fever is an oncologic emergency that requires immediate treatment with antibiotics.

Aim: To isolate and identify bacterial, fungal, parasitic and viral aetiological agents causing infections in febrile neutropenic children with haematological malignancy and to identify the co-morbid conditions.

Materials and Methods: This cross-sectional study was conducted at the Institute of Microbiology, Madras Medical College Chennai, Tamil Nadu, India, for a period of one year from November 2012 to October 2013. A total of 112 paediatric patients with 129 febrile episodes were included in the study satisfying the inclusion and exclusion criteria. Under strict aseptic precautions samples were collected from patients with signs of infections after obtaining informed consent and were subjected to microbiological detection and identification by conventional culture methods. The data was expressed in terms of frequency and percentages and statistical results were analysed with Microsoft Excel.

Results: Among the 129 febrile episodes microbiologically documented infections were 39.5% (51 out of 129 febrile episodes). Bacterial infections were predominant constituting 36.4% followed by 3.1% fungal infection. Bacteremia constituted to about 19.4% of febrile episodes, followed by urinary tract infection about 11.6%, respiratory tract infection 6.2% and oral infection 2.3%. Most of the gram negative isolates were sensitive to amikacin, cefaperazone sulbactam and piperacillin tazozobactam. Gram positive isolates were sensitive to vancomycin, cotrimoxazole and erythromycin. Out of the four fungal isolates three *Candida* spp. were isolated from oral thrush and one *Aspergillus flavus* from respiratory tract. All the fungal isolates were 100% sensitive to antifungal agents. Overall mortality due to infection was 18%.

Conclusion: In this study, the spectrum of bacteremia among febrile neutropenic patients, appear to be shifting towards gram positive microorganisms with multidrug resistant organisms being common. Emergence of multidrug resistance can be prevented by reviewing periodical modification of empiric antibiotic policy. Therefore, surveillance of antibiotic resistance pattern would be useful for deciding empiric therapy in them.

Keywords: Bacteremia, Bacteriological profile, Chemotherapy, Multidrug resistance

INTRODUCTION

Neutropenic fever is an oncologic emergency that requires immediate treatment with antibiotics. Neutrophils are the major cellular defence against most bacteria and chemotherapy induced neutropenia is well known to be associated with infections that may be life threatening. The pattern of organisms causing bacteremia is different, children experience more *Streptococcus* species in blood cultures, whilst adults had more *Staphylococcus* species [1]. The risk of infection in neutropenic patients increases rapidly when granulocyte count falls <500 cells/mm^3. Severe infection is observed when granulocyte counts are <100 cells/mm^3 [2]. The degree of neutropenia is directly related to the incidence of serious bacterial and fungal infection [3]. Children with haematological malignancies include (Acute Lymphocytic Leukaemia (ALL), Acute Myeloid Leukaemia (AML), Hodgkin's and Non Hodgkin's Lymphoma (NHL)) are subjected to severe and at times lethal infections. Infection remains the second most common cause of death and accounts for a large fraction of treatment related costs [4]. Gram negative bacilli are considered to be as the profound pathogen in patient undergoing immunosuppressive therapy whereas *Streptococcus viridans* which is gram positive cocci is found to be significant among neutropenic children [3]. Invasive fungal infections are also a serious risk for morbidity and mortality in this population. Availability of new antimicrobial agents has made it possible to treat infectious complications more effectively, but their availability is also leading to an increased prevalence of highly resistant pathogens. The overall incidence of infection in febrile neutropenic patients on chemotherapy is 0.25-0.5 episodes per patient per year and has increased upto 1.8 episodes per year in patients with advanced haematogical malignancies [5]. The spectrum of bacterial isolate has been changing considerably over the past four decades. In a review of various studies by European Organisation for Research and Treatment of Cancer (EORTC) it has been shown that the pattern of microorganisms isolated changes almost every 2-3 years [6]. Hence, it is advisable to study the pattern of infections and causative organisms at an interval of 2-3 years. So, this study was done to identify the microorganisms causing infections in febrile neutropenic children and to monitor the emerging resistant pathogens, which will help in the modification of empirical antibiotic therapy regimens.

MATERIALS AND METHODS

The present cross-sectional study was conducted at the Institute of Microbiology, Madras Medical College in association with Department of Paediatric Haematology, Institute of Child Health and Hospital for Children, Chennai, Tamil Nadu, India. This study was done for a period of one year from November 2012 to October 2013. Approval was obtained from the Institutional Ethical Committee (IEC) before the commencement of the study (No.18022013). Informed consent was obtained from the study population. All patients satisfying the inclusion

criteria were documented. Patients were interviewed by structured questionnaire. A total of 112 paediatric patients age less than 12 years diagnosed with various haematological malignancies (ALL, AML, Hodgkin's, Non hodgkin's lymphoma) on chemotherapy admitted as inpatients with fever and neutropenia and suspected of having infection based on the clinical symptoms and signs were taken for study.

Inclusion criteria [2]

1. Fever defined as a single oral temperature of 38.3°C or a persistent fever (temperature reading of 38°C on at least three consecutive evaluations (at >4 hours intervals) within 24 hours period.
2. Absolute Neutrophil Count (ANC) less than 500/mm^3 or a falling count anticipated to get less than 500/mm^3.
3. Patients who had repeated febrile neutropenic episodes during consecutive chemotherapy treatment were also enrolled in the study.

Exclusion criteria [2]

1. Those who developed fever within 24 hours after administration of chemotherapy and the fever subsided within next 24 hours after completion of chemotherapy.
2. Fever occurring during or within six hours of transfusion of blood, blood products and other intravenous fluids.

Study Procedure

Sample collection and processing: Under strict aseptic precautions samples including blood, sputum, resting gastric aspirate, urine, stool, Cerebro Spinal Fluid (CSF), tongue scrapings were collected from the patients and transported immediately to the laboratory in an appropriate settings. Processing of specimens included microscopy by gram staining, wet and iodine mount for stool, potassium hydroxide mount for tongue scrapings, wet mount, peripheral smear, giemsa staining for blood peripheral smear, Ziehl Neelsen (ZN) staining for sputum, modified acid fast staining for stool. Culture of the samples were done on suitable media like blood agar, MacConkey agar, Chocolate agar, Cysteine Lactose Electrolyte Deficient (CLED) agar, sabouraouds dextrose agar. After overnight incubation the samples which showed growth were subjected to relevant biochemical tests and susceptibility testing.

Antibiotic susceptibility testing: Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method. Antibiotics discs were obtained from Hi-media Laboratories (Mumbai, India) and were used as per manufacturer's instructions. Antibiotic susceptibilities of bacterial isolates were determined according to the method recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines [7].

Detection of MRSA, ESBLs and Carbapenemases: MRSA detection was done using cefoxitin disk and isolates showing zone diameter of ≤21 mm were considered as Methicillin Resistant *Staphylococcus aureus* (MRSA). Extended Spectrum Beta Lactamase (ESBL) detection was done using double disk diffusion method with combination disk of ceftazidime (CAZ 30 µg) and ceftazidime/clavulanic acid (CAZ/CA 30 µg/10 µg) >5mm increase in zone diameter for ceftazidime tested in combination with clavulanic acid versus its zone when tested alone is confirmed an ESBL producing organism. AmpC beta lactamase detection is done by cefotaxime (30 µg), ceftazidme (30 µg) disc which is placed adjacent to cefoxitin (30 µg) disc on a lawn culture of the organism at a distance of 20 mm from each other. After incubation, isolates showing blunting adjacent to cefoxitin disc were considered as positive for AmpC beta lactamases. Metallo Beta Lactamses (MBL) detection is done by imipenem-Ethylene Diamine Tetra Acetic Acid (EDTA) combined disc test. If the increase in inhibition zone with imipenem-EDTA disc is >7 mm than the imipenem disc alone, was considered MBL positive. Minimum Inhibitory Concentration (MIC) detection for imipenem and vancomycin was done by macrobroth dilution method. Antifungal susceptility were done with water insoluble drugs amphotericin-B, itraconazole and voriconazole by microbroth dilution method [7].

One CSF sample was subjected to Polymerase Chain Reaction (PCR) for diagnosis of Herpes Simplex clinical genomic Deoxyribonucleic Acid (DNA) mini prep kit, Biogene Inc., CA, USA as per the manufacturer's instructions. The amplified products were subjected to agarose gel electrophoresis.

STATISTICAL ANALYSIS

The data were collected and entered in Microsoft Excel 2013. Frequencies and percentages were determined for categorical variables.

RESULTS

The study population included children mostly in the age group 4-6 years. The most common malignancy among the study population was ALL followed by AML and NHL [Table/Fig-1]. Fever was accompanied by vomiting in 56 (43%) of patients [Table/Fig-2]. The distribution of febrile episodes was 129 episodes among 112 patients [Table/Fig-3]. In the present study, microbiologically documented infection was 51 (39.5%). Among the 129 febrile episodes bacterial infection constituted to about 47 (36.4%) of the febrile neutropenic episodes [Table/Fig-3]. In this study, bacteremia constituted to about 25 (19.4%) of febrile episodes, followed by urinary tract infection about 15 (11.6%) of febrile episodes, respiratory tract infection 8 (6.2%) and oral infection to 3 (2.3%) of febrile episodes [Table/Fig-4].

Type of haematological malignancies	Male	Female
ALL	55	34
AML	8	4
NHL	7	3
CML	0	1
Age group (years)	**Number**	**Percentage (%)**
1-3	23	20.5
4-6	39	34.8
7-9	35	31.3
10-12	15	13.4

[Table/Fig-1]: Sex distribution of children with febrile neutropenia (n=112).
ALL: Acute lymphoid leukaemia; AML: Acute myloid leukaemia; NHL: Non hodgkin's lymphoma; CML: Chronic myeloid leukaemia

Clinical features	Number	Percentage (%)
Fever	129	100
Vomiting	56	43
Dyspnoea	44	34
Cough	37	29
Dysuria	32	25
Mucositis/Oral thrush	30	23
Dysphagia	26	20
Abdominal pain	10	8
Diarrohea	6	5
Seizures	2	2
Headache	1	1

[Table/Fig-2]: Details of clinical presentation of patients with febrile neutropenia (n=129).

Number of febrile episodes	Number of patients	Total number of febrile episodes
1	98	98
2	11	22
3	3	9
Total	112	129

[Table/Fig-3]: Distribution of febrile episodes in febrile neutropenic children (n=129).

Sample	Aetiological agents				
	Bacterial isolates	Fungal isolates	Parasite	Virus	Total
Blood	25	-	-	-	25 (19.4%)
Urine	15	-	-	-	15 (11.6%)
Sputum	7	1	-	-	8 (6.2%)
Oral thrush	-	3	-	-	3 (2.3%)
Stool	-	-	-	-	-
CSF	-	-	-	-	-
	47 (36.4%)	4 (3.1%)	-	-	51 (39.5%)

[Table/Fig-4]: Details of aetiological agents in various samples during the febrile episodes by microbiological diagnostic methods (n=129).

The overall incidence of gram negative bacterial infection among the proven patients of infection was 26 (51%) and gram positive infection was 21 (41%). *Escherichia coli (E. coli)* was the predominant isolate constituting 13 (25.4%) followed by *Staphylococcus epidermidis* 11 (21.5%) [Table/Fig-5]. Among the 25 proven case of bacteremia, gram positive cocci contributed to 19 and gram negative bacilli found in six patients. Among the gram positive cocci causing bacteremia. *Staphylococcus epidermidis* was isolated among 11 patients and *Staphylococcus aureus* was isolated among eight patients. Among the gram negative bacilli causing bacteremia, *E. coli* was isolated in three patients, *Klebsiella pneumoniae* in two and *Pseudomonas* spp. in one [Table/Fig-5]. Most of the gram negative isolates were sensitive to amikacin, cefaperazone sulbactam and piperacillin-tazobactam [Table/Fig-6]. Among the gram positive isolates *Staphylococcus epidermidis* and *Staphylococcus aureus* showed (100%) sensitivity to vancomycin and were well within the sensitive range [Table/Fig-7]. In this study, prevalence of urinary tract infection was 11.6%, *E. coli* constituted to be the major isolate. The fungal agents isolated in this study were three *Candida* spp. from tongue scrapings and one *Aspergillus flavus* from sputum. Among the three *Candida* spp., one was found to be *Candida albicans* followed by one *Candida glabrata* and one *Candida tropicalis.* Antifungal susceptibility testing was done by microbroth dilution method as per CLSI guidelines. All the fungal isolates showed 100% sensitivity to amphotericin B, itraconazole and voriconazole by MIC microbrothdilution method, MIC being ≤1 for *Candida* spp., whereas *Aspergillus* MIC ≤2 for amphotercin B, itraconazole and voriconazole MIC ≤8. Infections related mortality was among 12 patients. Blood stream infections were predominant cause with drug resistant organisms [Table/Fig-8]. All the stool samples were subjected to wet mount and modified acid fast staining and were found to be negative. Blood samples were stained by Leishman's stain and examined for malarial parasites and were found to be negative. CSF PCR was done for one case of encephalitis and was found to be negative [Table/Fig-9].

Organism	Blood	Sputum	Urine	Oral thrush	Stool	CSF	Total	% among various group of organisms	% of Total
Gram positive cocci (n=21)							**21**		41
S. epidermidis	11						11	52	21.4
S. aureus	8	2					10	48	19.6
Gram negative bacilli (n=26)							**26**		51
E. coli	3		10				13	50	25.4
K. pneumoniae	2	3	3				8	30.8	15.6
K. oxytoca		1	1				2	7.7	4
Pseudomonas aeruginosa	1						1	3.8	2
Acinetobacter baumannii		1	1				2	7.7	4
Fungus (n=4)							**4**		8
Candida spp.				3			3	75	6
Aspergillus flavus		1					1	25	2
Total	25	8	15	3	-	-	51		100

[Table/Fig-5]: Distribution of gram positive cocci and gram negative bacilli isolates from various specimen.

	Escherichia coli (n=13)				*Klebsiella pneumoniae* (n=8)						*Klebsiella oxytoca* (n=2)				*Pseudomonas aeruginosa* (n=1)		*Acinetobacter baumannii* (n=2)			
	Blood n=3		Urine n=10		Blood n=2		Sputum n=3		Urine n=3		Sputum n=1		Urine n=1		Blood n=1		Sputum n=1		Urine n=1	
Antibiotics	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	3	100	8	80	2	100	3	100	3	100	1	100	1	100	0	0	0	0	1	100
Cefotaxime	1	33.3	7	70	1	50	-	-	2	66.6	-	-	1	50	0	0	0	0	1	100
Ceftazidime	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	1	100
Ciprofloxacin	1	33.3	5	50	1	50	1	33.3	1	33.3	-	-	1	50	0	0	0	0	1	100
Cefeperazone/ sulbactam	3	100	9	90	2	100	3	100	3	100	1	50	1	50	0	0	0	0	1	100
Gentamycin	1	33.3	1	10	1	50	-	-	1	33.3		-	-	-	0	0	0	0	0	0
Nitrofurantoin	-	-	6	60		-	-	-	1	33.3		-	1	50	-	-	-	-	1	100
Norfloxacin	-	-	5	50		-	-	-	1	33.3		-	1	50	-	-	-	-	1	100
Ofloxacin	3	100	-	-	1	50	1	33.3	-	-	0	0		-	0	0	0	0	-	-
Pipercillin-tazobactam	3	100	9	90	2	100	3	100	3	100	1	50	1	50	0	0	-	-	1	100
Imipenem (MIC)	3	100	10	100	2	100	3	100	3	100	1	100	1	100	0	0	1	100	1	100

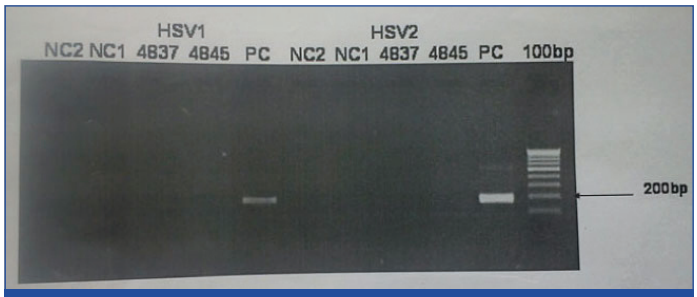
[Table/Fig-6]: Antimicrobial sensitivity pattern of the Gram Negative Bacilli (GNB).

Antibiotics	*Staphylococcus aureus* (n=10)				*Staphylococcus epidermidis*	
	Blood n=8		Sputum n=2		Blood n=11 [n (%)]	
	n	%	n	%		
Amikacin	6	75	1	50	11	100
Ciprofloxacin	3	37.5	1	50	4	36.3
Chloramphenicol	3	37.5	2	100	6	54.5
Cotrimoxazole	1	12.5	-	-	3	27.2
Erythromycin	3	37.5	1	50	5	45.5
Penicillin	3	37.5	-	-	4	36.3
Vancomycin (MIC)	8	100	2	100	11	100

[Table/Fig-7]: Antimicrobial sensitivity pattern of Gram Positive Cocci (GPC).

Total no. of patients	No. of patients died	No. of death in whom infectious aetiological agent was identified	Gram positive bacterial infection
112	12	9	3
Cause of death /Clinical diagnosis		**Specimen**	**Organism**
Case 1. Septicaemia		Blood	MRSA
Case 2. Septicaemia		Blood	MR *Staph. epidermidis*
Case 3. Septicaemia		Blood	*Klebsiella pneumoniae* (ESBL)
Case 4. Septicaemia		Blood	*E. coli*
Case 5. Pneumonitis with ARDS		Sputum	ESBL *Klebsiella pneumoniae*
Case 6. UTI with renal failure		Urine	*E. coli* (ESBL)
Case 7. Pneumonia with septicaemia		Sputum	*Staphylococcus aureus*
Case 8. Septicaemia		Blood	*Pseudomonas aeruginosa* (MBL)
Case 9. Pneumonitis with respiratory failure		Sputum	*Aspergillus flavus*
Case 10,11,12-Non infectious cause of death which is unknown			

[Table/Fig-8]: Infection related mortality in patients.
The other 3 cause of mortality is unknown



[Table/Fig-9]: Agarose gel electrophoretogram showing the amplification of HSV1 and HSV 2 targeting Glycoprotein D gene.

DISCUSSION

Infections in neutropenic children have remained a major clinical challenge over the present years due to many life threatening complications. There is a change in the spectrum of infections from gram negative to gram positive which may be due to long term use of prophylactic antibiotics. This has also increased the risk of antibiotic resistance. This study was conducted at a tertiary care centre in Chennai that treats especially haematological malignancies of children. Present study population included 98 patients with single episode of fever, 11 with 2 episodes and three with 3 episodes of fever. This constitutes to 1.2 episodes of infection per patient per year, it has been reported an overall incidence of 0.25-0.5 episodes of infection per patient per year and it could increase up to 1.8 episodes per year in patients with advanced disease [5,8].

In this study, fever was accompanied by vomiting in 56 (43%) of patients. Alcala-Chua MT also documented fever as the major symptom among these patients [9]; whereas Mahmud S et al., showed vomiting constituting (60%) of the presenting symptom among neutropenic children [2]. Presenting symptoms will give a clear picture of underlying infections and prompt investigations to be carried out. Among the specimen collected, blood and urine samples were collected from all 129 febrile episodes whereas other samples were collected from those patients with signs of organ specific infections. In the present study, microbiologically documented infection was 51 (39.5%) among the 129 febrile episodes. Dubey AP et al., in a study on site of infections and pathogenic organisms in childhood malignancies reported an infection rate of 36% in febrile neutropenic children with haematological malignancies [10]. According to a study clinically confirmed infection was 21% [11]. Bacterial infection constituted to about 36.4% of the febrile neutropenic episodes. This correlates with other study in similar population where infection rate was 46% among febrile neutropenic children with haematological malignancies [2]. In present study, bacteremia constituted to about 25 (19.4%) of febrile episodes, followed by urinary tract infection about 15 (11.6%) of febrile episodes respiratory tract infection 8 (6.2%) and oral infection to 3 (2.3%) of febrile episodes. This shows that bacteremia is the most common cause of infection this may be due to long term hospitalisation.

The overall incidence of gram negative bacterial infection among the proven patients of infection was 26 (51%) and gram positive infection was 21 (41%). *E. coli* was the predominant isolate constituting 13 (25.4%) followed and *Staphylococcus epidermidis* 11 (21.4%). According to Lv H et al., *E. coli*, CONS, Enterococci, *Klebsiella* were found to be the most frequent isolates [12] among paediatric haematooncology patients. Mahmud S et al., observed that *Staphylococcus aureus* was the most common isolate followed by *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [2]. Dubey AP et al., reported *E. coli* to be the most common isolate followed by *Klebsiella* species [10]. Burney IA et al., reported 46% of organisms isolated were gram positive [13]. Comparison of various studies showing the percentage of bacteremia in children is shown in [Table/Fig-10] [2,9,11,12,14].

Authors	Publication year	Place	Bacteremia (%)
Alcala-Chua MT [9]	1993	Philipines	28.9
Mahmud S et al., [2]	2004	Pakistan	25.5
Sayed HA et al., [11]	2009	Egypt	25
Phillips RS et al., [14]	2012	Meta-analysis report	17
Lv H et al., [12]	2012	China	43.48
Present study Dhanaleha P et al.,	2021	Chennai	19.4

[Table/Fig-10]: Comparison of various studies showing the percentage of bacteremia in children with febrile neutropenia.

In the present study, among the 25 proven case of bacteremia, gram positive cocci contributed to 19 (76%) and gram negative bacilli 6 (24%) this corresponds to the study by Lv H et al., who reported gram positive bacteremia in 55.7% and gram negative bacteremia 43.04%, respectively [12] among bloodstream infections in children with haematological malignancies. Mahmud S et al., reported gram positive bacteremia to be 56.3% and gram negative bacteremia to be 43.04% [2].

Among the gram positive cocci causing bacteremia *Staphylococcus epidermidis* was isolated in 11 (44%) of patients and *Staphylococcus aureus* was isolated in 8 (32%) of patients. This correlates with the increasing incidence of CONS [12]. Lv H et al., reported *Staphylococcus epidermidis* in 36.5% of bacteremia followed by *Staphylococcus aureus* 9.1% [12]. Among the gram negative baclli causing bacteremia, *E. coli* constituted 3 (12%), *Klebsiella pneumoniae* 2 (8%) and *Pseudomonas* 4% of patients. Lv H et al., observed *E. coli* in 15% followed by *Klebsiella pneumoniae* 15.19% and *Pseudomonas* 6.3% among a study on bloodstream infections in children with haematological malignancies [12]. Patient with gram negative bacteremia have a poor prognosis and higher mortality. Hence all regimens are chosen to combat gram

negative sepsis. This may explain the shift of gram negative bacteremia to gram positive bacteremia. Factors considered responsible for the shift towards gram positive isolates include aggressive chemotherapy, radiotherapy causing mucositis, profound and prolonged neutropenia, unrecognised herpetic infection of the mucous membrane, increased use of long indwelling intravenous catheters and use of newer quinolones in neutropenic patients [5]. Patients in present study had long term neutropenia with aggressive chemotherapy with cydarabin.

Among febrile neutropenic children bacterial infections are the most common cause of illness and death. In this study, prevalence of urinary tract infection was 15 (11.6%), *E. coli* constituted to be the major isolate followed by *Klebsiella pneumoniae*. Sandoval C et al., showed among paediatric oncology patients with neutropenia and the frequency of urinary tract infection to be 8.6% [15]. A similar study by Munyi ST et al., showed 8.1% of urinary tract infection among these patients and the most common organism was found to be *E. coli* [16].

Sputum and resting gastric aspirates from patient with lower respiratory tract infection were subjected to culture, out of the 11 specimens collected, eight showed culture positivity five were gram negative bacilli, two were gram positive cocci and one was a fungal isolate, which constitutes to 6.3% of febrile episodes. This was in contrast to the study by Dubey AP et al., who observed 26% respiratory tract infections and Sayed HA et al., reported a higher rate of 23% of respiratory tract infections among similar group [10,11]. This can be explained as out of 37 patients having cough only eight patients were able to give sputum sample or gastric aspirate.

Antimicrobial susceptibility testing was done as per CLSI guidelines. Most of the gram negative isolates were sensitive to amikacin, cefaperazone sulbactam and piperacillin-tazobactam. Among the mechanism of resistance ESBL production was the most common. The isolates were confirmed by double disc synergy test. Most of the ESBL producer was isolated from blood followed by urine and sputum. According to Butt T et al., an incidence of ESBL-producing *Klebsiella pneumoniae* among febrile neutropenic paediatric patients were found to be 51.6% among blood isolates [17]. One *E. coli* isolate from urine was found to be AmpC producer. One isolate of *Pseudomonas aeruginosa* from blood and one *Acinetobacter baumannii* isolate from sputum were found to be MBL positive by imipenem and imipenem-EDTA combined disc method. Butt T et al., reported multidrug resistance among *Pseudomonas aeruginosa* in 34.1% among blood stream infections in paediatric haematological malignancy patients [17]. Among the gram positive isolates *Staphylococcus epidermidis* and *Staphylococcus aureus* showed 100% sensitivity to vancomycin and were well within the sensitive range. Among the gram positive cocci isolated from blood methicillin resistance were seen among, 40% of *Staphylococcus aureus* and 36.4% of *Staphylococcus epidermidis*.

This may be due to inadequate antibiotics or longer duration of hospitalisation, which may selectively enhance the growth of multidrug resistant organisms. In a similar study on blood stream infections among paediatric haematological malignancies, methicillin-resistance was observed among 90% of staphylococcal isolates [18]. Paya E et al., in a similar study showed a significant increase of 55% CONS resistant to methicillin and 44% by *Staphylococcus aureus* among blood isolates [19]. According to Mahmud S et al., and Ammann RA et al., *E. coli*, *Pseudomonas* and *Klebsiella* showed greater degree of drug resistance to the commonly used antibiotics. Among gram positive cocci *Staphylococcus aureus* and *Staphylococcus epidermidis* were resistant to penicillin [2,20]. Karim M et al., in 1991 showed nosocomial isolates of *Pseudomonas* aeruginonosa had high frequency of antibiotic resistance as compared to community acquired infection [21]. In a study by Dubey AP et al., documented 100% sensitivity to amikacin, ciprofloxacin, cefotaxime and penicillin [10].

Microbiologically, proven fungal infection in this study constituted to about 3.1% of the infectious episodes. The fungal agents isolated in this study were three *Candida* spp. from tongue scrapings and one *Aspergillus flavus* from sputum. Sayed HA et al., reported *Aspergillus* spp. in 15% of respiratory tract infections among paediatric haematology patients [11]. In this study, oral thrush was observed in three patients with oral thrush out of which all patients were culture positive for *Candida* spp. A study by Michaud M et al., showed an incidence of 21% candidiasis in the presence of oral lesions [18]. Among the three *Candida* spp. one was found to be *Candida albicans* followed by one *Candida glabrata* and one *Candida* tropicalis. According to Michaud M et al., among oral Candidiasis in paediatric leukaemia patients the most common *Candida* spp. was *Candida albicans* [18]. In a study by Sayed HA et al., showed non albicans *Candida species* were more predominant than *Candida albicans* as overall cause of fungal infections among which *Candida parapsilosis* and *Candida tropicalis*, accounting for 25.5% and 23.6%, respectively [11]. Antifungal susceptibility testing was done by microbroth dilution method as per CLSI guidelines. All the fungal isolates showed 100% sensitivity to amphotericin B, itraconazole and voriconazole by MIC method.

In the present study, out of 51 cultures proven patients nine patients died due to infection related complications which accounts for 18% mortality rate. Cause of death was due to sepsis in most of the patients. Mahmud S et al., Dubey AP et al., Naqvi SMA et al., observed infection related mortality rate of 22%, 27%, and 22.7% respectively among paediatric haematological malignancy patients [2,10,22]. Septicaemia was observed in five patients whereas pneumonia, urinary tract infection along with other complication was observed in rest of the patient. In this study, one patient died due to fungal pneumonitis caused by *Aspergillus flavus* which shows invasive fungal infection is the major cause of morbidity and mortality in neutropenic patients. Mortality due to fungal infection was 1%. This study will give knowledge about the empirical antibiotics to be started with the present antibiotic susceptibility pattern and also the various clinical outcomes of the patients. Since very few places deal exclusively in children haematological malignancies, this will contribute further in doing more studies in future which deals with infections.

Limitation(s)

Since, the study was for a short period detailed follow-up of the patients could not be done. But further increasing the time period will give a clear picture of clinical outcomes. Moreover molecular methods of detecting multidrug resistance could not be done which could have added more value to the study.

CONCLUSION(S)

In this study, the spectrum of bacteremia among febrile neutropenic patients, appear to be shifting towards gram positive microorganisms. Multidrug resistance due to ESBL, AmpC and MBL production was observed among Enterobacteriaceae and non fermenter gram negative bacilli. All the fungal isolates were susceptible to the antifungal agents. Mortality was higher among patients with bacteremia due to multidrug resistant organisms than due to antibiotic susceptible organisms. Emergence of multidrug resistance can be prevented by reviewing periodical modification of empiric antibiotic policy. Multidrug resistance can be life threatening in such children. Therefore, surveillance of antibiotic resistance pattern would be useful for deciding empiric therapy in them.

Acknowledgement

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references to this manuscript. The authors are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Authors' contribution: Dr P. Dhanaleha: Clinical study, Dr Anand MR: Sample collection, manuscript writing and editing, Dr. G. Jayalakshmi: Clinical study and manuscript writing.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 19, 2021
- Manual Googling: Nov 03, 2021
- iThenticate Software: Nov 05, 2021 (24%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Apr 17, 2021**
Date of Peer Review: **Jul 02, 2021**
Date of Acceptance: **Nov 06, 2021**
Date of Publishing: **Dec 01, 2021**