

Original Research Article

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Microbiological Profile of Blood Stream Infection in Neutropenic Patient in a Tertiary Care Centre

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ABSTRACT

Blood stream infection is a major cause of morbidity and mortality among neutropenic patients. Despite use of antibiotics and antifungals FN remains a therapeutic challenge. It prolongs hospital stay, increases health-care costs, and compromises chemotherapy efficacy in patients with malignancy who commonly experience neutropenia. This study depicts the microbiological profile, antibiotic sensitivity pattern and relation of BSI and degree of neutropenia in the neutropenic patients. The study was a hospital based prospective observational study conducted in a tertiary care centre in North east, India. Correlation of degree of neutropenia and BSI with analysis of microbiological profile and antibiotic sensitivity pattern of the FN patients admitted from September 2014 to September 2016 was done. A total of 259 cases of fever with neutropenia were included from September 2014 to September 2016 in the present study. Of these, 55 (21.33%) were found to be culture positive. Majority of case 79 (30.5%) were suffering from Acute Myeloid Leukemia (AML). Gram negative bacilli 58.18 % (32) is the most common isolate. The antibiotic sensitivity among GNB was highest for colistin (100%) and teigecycline (93.8%). There is an alarming increase in resistance for cephalosporins and Carbapenems. The frequency of isolation of micro organisms in blood by blood culture in neutropenic patient with fever is 21.23% and Gram negative bacilli is still the predominant pathogens. There is an alarming rise in the resistance against carbapenems and commonly used drugs. It needs to be emphasized that a routine microbiological examination of these group of patients should be carried out routinely and periodically so as to analyse and compare the changing trends in the microbial aetiology and the antibiogram patterns. It is recommended that an effective empirical antibiotic regime could be tailored for this group of patients to decrease the morbidity and mortality.

Keywords

Blood Stream Infection, Neutropenic Patient.

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Introduction

Blood stream infection is a major cause of morbidity and mortality among neutropenic patients (Tariq *et al.*, 2002; Honar *et al.*, 2003). Despite use of antibiotics and antifungals FN remains a therapeutic challenge. It increases health-care costs, prolongs hospital stay and compromises chemotherapy efficacy, due to delays and dose reductions. Susceptibility to infectious

diseases increases sharply when neutrophil counts fall below 1000 cells/ μ L. Acute neutropenia, such as that caused by cancer chemotherapy, is more likely to be associated with increased risk of infection than neutropenia of long duration (months to years) that reverses in response to infection or carefully controlled administration of endotoxin (Steven *et al.*).

In about 20 – 30 % of the episodes of neutropenic fever, bacteremia is diagnosed. The crude rate for blood stream infections (BSI) in cancer patients ranges from 18 to 42% (Ehni *et al.*, 1991; Prabhash *et al.*). While the incidence of gram-negative infections (71%) was higher than gram-positive (29%) in the 1960s and 1970s, increased use of indwelling catheters, early-generation quinolone prophylaxis, and broad-spectrum empirical anti gram-negative antibacterial therapy led to an increase in the incidence of gram-positive pathogens (69%) in the 1980s and 1990s (Alison *et al.*, 2010; Schimpif, 1991; Reuben *et al.*, 2004). Honar Cherif *et al.*, (2003) in their 14 years study found Coagulase Negative *Staphylococci* (17%) being the dominating pathogen. Akihisa kanamaru *et al.*, (2004) found Gram negative organisms were more prevalent during 1985-1996, with pseudomonas species being the commonest but Gram positive organisms were more prevalent, with staphylococcus species dominance during 1997-2002. Anaerobes are infrequent cause. In an Asian study by Tariq Butt *et al.*, no anaerobic organisms were isolated.

Febrile neutropenia (FN) is one of the major causes of morbidity and mortality in patients of hematological malignancies. These patients may not present with any obvious source of infection except blood stream infection. Hence, in all patients suspected of FN, blood culture sensitivity should be sent if possible before the first dose of antibiotics. These patients generally have associated anaemia and thrombocytopenia (Arun *et al.*, 2013).

There has been a considerable change in spectrum of pathogens causing infection in neutropenic patients. Prabash *et al.*, in a retrospective study, in India in the year 2007 found high degree of resistance to the cephalosporins with only 27.1% of the Gram Negative isolates being sensitive to third

generation cephalosporins, namely ceftriaxone and cefotaxime. Meropenem was the most effective antibiotic, active against 71.7% of the Gram negative isolates. The antibiotic most active against Gram positive was linezolid. Because the progression of infection in neutropenic patients can be rapid, and because such patients cannot be reliably distinguished from non-infected patients upon presentation, empirical antibiotic therapy should be administered promptly to all neutropenic patient at the onset of fever. It is administered with the goal of eradicating the most frequent organisms causing fulminant infections, which may result in serious complications. In the setting of changing flora and susceptibility patterns to antibiotics, guidelines as to “best therapy” of infection in the neutropenic patient must be evaluated on the basis of local patterns of infection and local and regional resistance patterns

So there is a need to know the local pattern of organisms causing bacteremia, so that an effective empirical antibiotic therapy can be formulated for the neutropenic patients to reduce the mortality and morbidity rate. Keeping this in view, the present study was carried out in a tertiary care centre to know the frequency of bacteremia in neutropenic patient, to isolate the organisms and study the antibiotic sensitivity pattern causing bacteremia in neutropenic patients.

Materials and Methods

The study was a hospital based prospective observational study conducted in a tertiary care centre. Analysis of microbiological profile and Antibiotic sensitivity pattern of the FN patients admitted from September 2014 to September 2016.

259 cases presenting with Fever and Absolute neutrophil count $< 500/\text{mm}^3$ (Fever was defined as single oral temperature of $\geq 38.3^{\circ}$)

Celsius (101° F) or a temperature of $\geq 38^{\circ}$ Celsius (100.4° C) for more than 1 hour were included in the study. Patients who became febrile in proximity to having received blood products and Patients not willing to undergo the procedure were excluded from the study.

History was obtained and physical examination was performed. Blood was drawn for blood culture before empirical antimicrobial therapy was started or just before the next dose of antibiotic. One specimen of blood for aerobic and anaerobic cultures was drawn through peripheral vein. A second sample was collected at the same day from a different venipuncture to rule out contamination with skin flora.

Immediately after inoculation, VersaTREK Blood culture (Redox1, Redox2) bottles were incubated in the VersaTrek. Subculture was done from the incubated broths on 10% sheep Blood Agar, MacConkey's Agar and Chocolate Agar when flagged positive. A smear was also made from the broth after inoculation and gram stain was done. The smear was examined for presence of any microorganisms.

The inoculated Blood Agar and MacConkey's Agar media plates were incubated at 37° C for 24 hours aerobically. The inoculated Chocolate Agar plates were kept in Oxoid Anaerobic jar with the anaerobic gas pack (HIMEDIA) and anaerobic indicator, and incubated at 37° C for 24 hours. Characterization and identification of organisms was done as per Collee *et al.*, (1996).

Biochemical identifications of the isolates were carried out by conventional methods and VITEK 2 compact system.

All the isolated bacteria were tested against different antimicrobial agents by the Vitek2

as well as the standard disc diffusion method (Kirby Bauer Technique). Commercially available AST kits AST-P628 (REF 414534) for Gram Positive cocci and AST-N281 (REF 414532) for Gram Negative bacilli were used from biomerieux. For disc diffusion method commercially available antibiotic discs were used obtained from Hi Media Laboratories Limited.

Results and Discussion

A total of 259 cases of fever with neutropenia were included from September 2014 to September 2016 in the present study. Blood samples were collected from the various department and Intensive care units (ICU) of Gauhati medical college. Bacteriological studies of the specimens were carried out in the department of microbiology, Gauhati Medical College and Hospital for a period of 2 years. Blood cultures from neutropenic patients with fever were collected and processed, with maximum patients from Hematology department 189 (73%) and M: F ratio 1.7:1. Of these, 55 (21.33%) were found to be culture positive. Highest number of cases was in the younger age group.

Majority of case 79 (30.5%) were suffering from Acute Myeloid Leukemia (AML), followed by 73 (28.2%) Acute Lymphocytic Leukemia (ALL) (Table 1). Table 2 indicates that the occurrence of BSI is directly proportional to the Absolute Neutrophil count, with highest 15 (88.23%) positive Blood culture in patients with ANC $< 100/\mu\text{l}$. Out of the 259 neutropenic patients only 53(21.23%) were blood culture positive. However no anaerobic organisms were isolated. 2 isolates of *Candida tropicalis* were isolated (Table 3). Gram negative bacilli 58.18 % (32) is the most common isolate followed by 38.18% (21) Gram positive cocci (Table 4).

Among the Gram negative isolates *Escherichia coli* 50% (16) is the predominant isolate followed by *Klebsiella pneumoniae* 43.8 % (14) and *Acinetobacter baumannii* complex 6.25 % (2) (Table 5). A total of 38.18 % (21) Gram positive cocci were identified of which 47.61% (10) were Coagulase negative *Staphylococci* (CONS), the predominant organism isolated among GPC, 28.57% (6) were *Staphylococcus aureus*, and 5 (23.8 %) were of *Enterococcus* species (Table 6). 2 MRSA was also detected among the isolates.

The antibiotic sensitivity among GNB was highest for Colistin (100%) and Teigecycline (93.8%). There is an alarming increase in resistance for cephalosporins and carbapenems (Table 7). The GPC were sensitive to Daptomycin, Teigecycline, Vancomycin, Teicoplanin and Linezolid. However they were highly resistant to Fluroquinolones and Clindamycin (Table 8 and Fig. 1).

In our study we observed that the Gram positive isolates were 100% sensitive to Teigecycline and Daptomycin followed by Linezolid, Vancomycin and Teicoplanin with 90.5% sensitive. Whereas clindamycin was 100% resistant followed by ciprofloxacin, levofloxacin and erythromycin with 66.7, 66.7% and 76.2% resistance respectively. MRCoNS are a concerning issue as they are resistant to many drugs.

Patients with malignancies and neutropenia are at high risk for the development of Blood stream infection (BSI). The causative organisms have changed over time. The Gram-negative bacteria were the main responsible for the febrile neutropenia in the sixties; their impact declined due to the use of fluoroquinolone prophylaxis. This situation was followed by the gradual emergence of Gram-positive bacteria also following the

increased use of intravascular devices and the introduction of new chemotherapeutic strategies. In the last decade, the Gram-negative etiology is raising again because of the emergence of resistant strains that make questionable the usefulness of current strategies for prophylaxis and empirical treatment.

This necessitates a periodic monitoring of the locally prevalent pathogens and their antibiograms, so that a rational empiric antimicrobial therapy for neutropenic patients may be formulated.

In the present study, out of 259 cases, highest number of case 73% were from Haematology department and majority of cases 30.5% were suffering from Acute Myeloid Leukemia (AML), followed by 28.2% Acute Lymphocytic Leukemia (ALL). Similar results were obtained in other studies, Michelle Karim *et al.*, (2015) with 42% cases having AML, followed by 12% ALL. Lakshmaiah *et al.*, (2015) 2011 – 2013, also reported AML (55 episodes) as the most common etiology. The high incidence of incidence of febrile neutropenia in AML could be due to the use of intensive chemotherapy leading to prolonged and profound neutropenia, thus increasing the risk of infection.

Our study reveals that the rate of blood culture positivity is highest (88.23%) in those patients with ANC <100/ μ l and only 13.04% in ANC >200/ μ l. Similar relation was established by Bodey *et al.*, (1966) according to the study the frequency of severe infection is highest when absolute neutrophil count was 100/ μ l and proportionately less frequent at ANC 100 – 500/ μ l and ANC 500-1000/ μ l. Only 21.23% (53) has shown the growth in blood culture. Similar results were reported by Lakshmaiah *et al.*, 2011 – 2013, in South India 19.44% positive blood culture in neutropenic patients. But in a study Mandal *et*

al., (2015) 2010-2013 in a similar study in India found 29.1% culture positivity. However no anaerobic organisms were isolated in our study, which is in conformity with other similar studies done by Tariq Butt *et al.*, 2002. However Honar Cherif *et al.*, 1988-2001 found 4.1% anaerobic isolates. The positivity rate can be probably explained by the observation made by Matsuhisa *et al.*, (1994) they observed in febrile neutropenic patients most bacteria are phagocytized by neutrophils and only few remain in blood to form colonies detectable by culture thus emphasizing the use of in situ hybridization to detect bacterial DNA.

Our study shows Gram negative bacilli 58.18% (32) is the most common isolate followed by 38.18 % (21) Gram positive cocci. Similar observation was made by other workers in India, Lakshmaiah *et al.*, 2011 – 2013, reported, GNB 57.14% (12) as predominant isolate and GPC 42.86% (9). Mandal *et al.*, (1994) 2010-2013 reported

GNB 61.53% (48) as the predominant isolate followed by GPC 34.61% (27). Dong –Gun Lee *et al.*, (2011) mentioned that as a general characteristic of Asia-Pacific region, Gram-negative microorganisms were the major pathogens of infection.

Among the 32 Gram negative isolates *Escherichia coli* 50% (16) is the predominant isolate followed by *Klebsiella pneumoniae* 43.75% (14) and *Acinetobacter baumannii* complex 6.3% (2). Tariq Butt *et al.*, 2002, reported 13.25% (11) *E. coli* as the predominant isolate. A total of 38.18 % (21) Gram positive isolates were identified of which 47.61% (10) were coagulase negative *Staphylococci*, the predominant organism, 28.57 % (6) were *Staphylococcus aureus*, and 23.8 % (5) were of *Enterococcus* species isolated among the GPC. Similar results were seen by Tariq Butt *et al.*, (2002), most common isolates were CONS 26.5% (22), Prabhash *et al.*, (2007), CONS 10.54% (51) among GPC isolates.

Table.1 Distribution of total diagnosed cases

Diagnosis	No.	Percentage
Solid tumors	21	8.1
Acute Myeloid Leukemia	79	30.5
Acute Lymphocytic Leukemia	73	28.2
Chronic Lymphocytic Leukemia	5	1.9
Chronic Myeloid Leukemia	15	5.8
Non - Hodgkin's Lymphoma	13	5
Hodgkin's Lymphoma	3	1.2
Myelo Dysplastic Syndrome	8	3.1
Acute leukemia	21	8.1
Aplastic anaemia	10	3.9
Hypoplastic anaemia	3	1.2
Multiple myeloma	5	1.9
Langerhan's cell histiocytosis	3	1.2

Table.2 Relation of ANC and culture positive

ANC (μl)	No. of FN episodes	NO.(%) Of positive Blood culture
< 100	17	15 (88.23%)
100 - 200	35	13 (37.14%)
201 - 500	207	27 (13.04%)

Table.3 Total blood culture positive cases

Total no. of Blood Culture	No. of culture positive cases	percentage
259	Aerobic Bacteria	53 20.46%
	Anaerobic Bacteria	0 0%
	Fungus	02 0.77%

Table.4 Isolates in total positive blood culture

Pathogens	Total No. of isolates	percentage
GPC	21	38.18%
GNB	32	58.18 %
Fungus	2	3.64%
Total	55	100%

Table.5 Different isolates of Gram negative organisms

Gram Negative Bacilli	No.	Percentage
<i>Escherichia coli</i>	16	50 %
<i>Klebsiella pneumoniae</i>	14	43.8 %
<i>Acinetobacter Spp.</i>	2	6.25 %
Total isolates	32	100%

Table.6 Different isolates of Gram positive cocci

Gram positive cocci		No. of isolates	
<i>Staphylococcus aureus</i>		6 (28.57%)	
Coagulase Negative <i>Staphylococci</i>	<i>Staphylococcus hemolyticus</i>	6	10 (47.6%)
	<i>Staphylococcus saprophyticus</i>	4	
Enterococci	<i>Enterococcus faecalis</i>	4	5 (23.8%)
	<i>Enterococcus faecium</i>	1	
Total cases		21(100 %)	

Table.7 Sensitivity pattern of GNB isolates

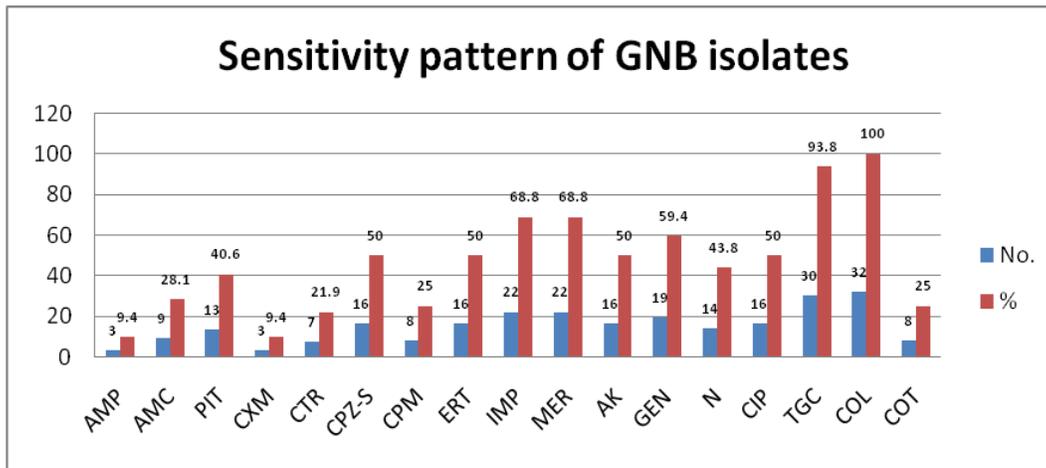
ISOLATES	MDR%	PDR%	VA Resistance%
<i>Staphylococcus aureus</i>	16.7	0	33.3
CONS	20	0	0
Enterococci	0	0	0

Table.8 Sensitivity of gpc isolates

Isolates of GPC		CX	P	OX	GEN	CIP	LEVO	CD	E	LZ	DAP	TEI	VA	TET	TGC	RIF	COT
<i>Staphylococcus aureus</i> n= 6	No.	4	2	4	4	2	2	0	2	6	6	4	4	6	6	4	4
	%	66.7	33.3	66.7	67	33	33.3	0	33	100	100	67	66.7	100	100	66.7	66.7
CONS n= 10	No.	0	0	0	10	5	5	0	0	10	10	10	10	5	10	3	10
	%	0	0	0	100	50	50	0	0	100	100	100	100	50	100	30	100
Enterococcus n= 5	No.		0		3	0	0		3	3	5	5	5	0	5		
	%		0		60	0	0		60	60	100	100	100	0	100		
Total n= 21	No.				17	7	7	0	5	19	21	19	19	11	21	7 (n=16)	14 (n=16)
	%				81	33.3	33.3	0	23.8	90.5	100	90.5	90.5	55.6	100	43.8	87.5

P= penicillin, OX= Oxacillin, E= Erythromycin, GEN= Gentamicin, LEVO= Levofloxacin, CD= Clindamycin, LZ= Linezolid, DAP= Daptomycin, TEI= Teicoplanin, VA= Vancomycin, TET= Tetracycline, TGC= Teigecycline, RIF= Rifampicin, COT= Cotrimoxazole, CX= Cefoxitin

Fig.1 Sensitivity pattern of GNB isolates



AMP=Ampicillin, AMC= Amoxycillin + clavulanic acid, PIT= Piperacillin + Tazobactam, CXM= Cefuroxime, CTR=Ceftriaxone, CPZ-S=Cefoperazone + sulbactam, CPM=Cefepime, ERT=Ertapenem, IMP=Imipenem, MER=Meropenem, Ak=amikacin, GEN=Gentamicin, N= Nalidixic acid, CIP= Ciprofloxacin, TGC=Teigecycline, COL= Colistin, COT=Cotrimoxazole

In this study we observed that the 32 Gram negative bacilli isolated were 100% sensitive to Colistin, followed by 93.8 % sensitive to Teigecycline and 68.8% sensitive to Imipenem and Meropenem. However contrasting our findings

Kuntegowdanahalli C Lakshmaiah *et al.*, 2011-2013, found imipenem 100% sensitive, followed by Piperacillin-tazoactum 86.95% sensitive. Tariq Butt *et al.*, 2002, also found imipenem to be 100% sensitive.

In this study it was observed that the Gram positive isolates were 100% sensitive to teigecycline and daptomycin followed by linezolid, vancomycin and teicoplanin with 90.5% sensitive. Whereas clindamycin was 100% resistant, the antimicrobial susceptibility pattern found is similar to other workers, Tariq Butt *et al.*, 2002 reported vancomycin and Imipenem to be 100% sensitive and Ampicillin to be 88.9% resistant. Prabhash *et al.*, 2007, Linezolid was 100% sensitive.

In conclusion, the findings of the present study revealed that the frequency of isolation of bacteria in blood by blood culture in neutropenic patient with fever is 21.23% and Gram negative bacilli is still the predominant pathogens.

The antibiograms revealed that the Gram positive cocci were 100% sensitive to teigecycline and daptomycin but resistance to commonly used drugs are at a rise.

The Gram negative bacilli isolated were 100% sensitive to colistin and 93.8 % to teigecycline, but there is an alarming rise in the resistance against carbapenems and commonly used drugs.

As the data of antibiogram of the isolates from neutropenic patients is sparse in this region and as seen in our study a fall in neutrophil count < 100 makes the individual vulnerable to infection by microorganisms so it needs to be emphasized that a routine microbiological examination of these group of patients should be carried out routinely and periodically so as to analyse and compare the changing trends in the microbial aetiology and the antibiogram patterns. So that an effective empirical antibiotic regime could be tailored for this group of patients to decrease the morbidity and mortality.

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