Gram Negative Bacilli Causing Blood Stream Infection in Febrile Neutropenic Patients in a Tertiary Care Centre

Joydeep Mangaraj[1], Dipa Barkataki[2], Daiji Gogoi Mohan[3]

ABSTRACT

Introdoction: The alarming rise in the incidence of infections due to antibiotic-resistant gram-negative bacilli in recent years in hospital setting is of great concern, a situation compounded in neutropenic patients, as they may initially receive antibiotics that are resistant. The present study depicts the frequency of Gram negative bacteremia in neutropenic patients, burden of MDR and PDR Gram negative bacilli, antibiotic sensitivity pattern of the isolated organisms and relationship between ANC (Absolute Neutrophil Complete) and BSI in neutropenic patients.

Material and methods: It is a hospital based prospective study conducted in a tertiary care centre Guwahati. Analysis of microbiological profile and Antibiotic sensitivity pattern of the FN patients admitted from September 2014 to September 2016 was done. All the isolated bacteria were tested against different antimicrobial agents by the Vitek2 as well as the standard disc diffusion method (Kirby Bauer Technique). For isolates of Gram negative Bacilli Extended-spectrum β-lactamase (ESBL) production was screened for by testing ceftazidime and cefotaxime, and confirmed with the double-disk test.

Result: The occurrence of BSI is inversely proportional to the Absolute Neutrophil count, with highest 15 (88.23%) positive Blood culture in patients with ANC < 100/µl. Gram negative bacilli is the most common isolate with a share of 58.18% (32) of the positive cases. The antibiotic sensitivity among GNB was highest for Colistin (100%) and Teigecycline (93.8%). There is an alarming increase in resistance for cephalosporins and carbepenems. 64.3% of Klebsiella pneumoniae and 37.5% of Escherichia coli were found to be Multi Drug Resistant (MDR).

Conclusion: Gram negative bacilli is the predominant pathogen with a share of 58.18% (32) of the positive cases in this region causing BSI in FN patients. BSI is inversely proportional to the Absolute Neutrophil count in neutropenic patients. There is an alarming rise in MDR organisms and resistance against carbapenems. It needs to be emphasized that microbiological examination of these group of patients should be carried out routinely and periodically, so that an effective empirical antibiotic regime could be tailored for these patients against the commonly causative organisms.

Keywords: Gram Negative Bacilli, Blood Stream Infection, Febrile Neutropenic Patients

INTRODUCTION

The alarming rise in the incidence of infections due to antibiotic-resistant gram-negative bacilli in recent years in hospital setting is of great concern, a situation compounded in neutropenic patients, as they may initially receive antibiotics that are resistant. One of the major cause of morbidity and mortality among neutropenic patient is Blood stream Infection, despite use of antibiotics,[1,2] making it a therapeutic challenge. It increases health-care costs, and compromises chemotherapy efficacy, due to prolong hospital stay and delay in dose reduction. There is increased Susceptibility to infectious diseases when neutrophil counts decreases below 1000 cells/µL. There is increased risk of infection in Acute neutropenia, such as that caused by cancer chemotherapy, than neutropenia of longer duration that responds to controlled administration of endotoxin.[3]

Bacteremia is diagnosed in about 20 – 30% of the episodes of neutropenic fever.[2] The incidence of GN infections (71%) was higher than gram-positive (29%) in the 1960s and 1970s, in 1980s and 1990s[4,5] increased use of indwelling catheters and empirical broad-spectrum antibacterial therapy led to an increase in isolation of gram-positive organisms (69%). Gram negative organisms were more prevalent during 1985-1996, Akihisa kanamaru et al[6], but during 1997-2002 Gram positive organisms were more prevalent. Anaerobes are not a common cause of BSI, Tariq Butt et al.[1] In Hematological malignancies, Febrile neutropenia (FN) is one of the major causes of morbidity and mortality. These patients present with BSI without any obvious source of infection, making it essential to send blood culture sensitivity in all patients suspected of FN before the first dose of antibiotics.

The spectrum of pathogens causing infection in neutropenic patients has been seen to be changing considerably. In a retrospective study, in India, cephalosporins were found to be highly resistant with only 27.1% GNB sensitive to ceftriaxone and cefotaxime.[7]. Meropenem was found to be 71.7% sensitive for the Gram negative isolates. There Infection progresses rapidly in neutropenic patients, so empirical antibiotic therapy should be administered to all neutropenic patients at the onset of fever promptly. It is administered with the goal of eradicating the most frequent organisms causing fulminant infections, which may result in serious complications. Administration of antimicrobial therapy at the earliest is an important determinant of survival, especially in these patients. An overall reduction (11%) in

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crude mortality rate was associated with adequate early empirical antimicrobial therapy and initial antimicrobial therapy with resistant antibiotics was identified as a risk factor for mortality.[8] In this scenario of changing flora and susceptibility patterns to antibiotics, certain guidelines for the "best therapy" of infection in the neutropenic patient must be tailored on the basis of local patterns of infection and local and regional resistance patterns to reduce the mortality and morbidity rate. Keeping this in view, the present study was carried out in the Department of Microbiology, to know the frequency of Gram negative bacteremia in neutropenic patient, to study the antibiotic sensitivity pattern of the isolated organisms and relationship between ANC (Absolute Neutrophil Complete) and BSI in neutropenic patients.

MATERIAL AND METHODS

It is a hospital based prospective study conducted in a tertiary care centre Guwahati. Analysis of microbiological profile and Antibiotic sensitivity pattern of the FN patients admitted from September 2014 to September 2016 was done. Blood samples were collected from the FN patients from various department and Intensive care units (ICU) of a tertiary care centre bacteriological studies of the samples from patient with BSI were carried out in the department of microbiology, for a period of 2 years. Cases fulfilling the inclusion criteria i.e. patients presenting with Fever and Absolute neutrophil count < 500/mm^3 (Fever defined as single oral temperature of ≥ 38.3^0 Celsius (101^0 F) or a temperature of ≥ 38^0 Celsius (100.4^0 C) for more than 1 hour[9]) were included. Patients not willing to undergo the procedure and who became febrile in proximity of receiving blood products were excluded from the study. For blood culture, blood was collected before empirical antimicrobial therapy was administered or just before the administration of next dose of antibiotic after filling the preformed proforma with consent. One blood specimen for aerobic and anaerobic each was drawn through the peripheral vein. From a different venipuncture site a second sample was collected on the same day. The blood collected and after inoculation, VersaTREK Blood culture (Redox1, Redox2) bottles were incubated in the VersaTrek. Then Subculture was done from the incubated broths on MacConkey's Agar, 10% sheep Blood Agar media and Chocolate Agar media when flagged positive by the machine. We then made a smear also from the broth after inoculation and gram stain was done and reported. The smear was examined for the presence of microorganisms. The streaked MacConkey and Blood Agar Agar media plates were incubated aerobically at 37^0 C for 24 hours. Chocolate Agar plates were kept in Oxoid Anaerobic jar with the Anaerobic gas pack (HIMEDIA) and anaerobic indicator, and incubated at 37^0 C for 24 hours. Characterization and identification of organisms was done as per Collee et al.[10] (1996),

Bacteria isolated were tested against different antimicrobial agents by the the standard disc diffusion method (Kirby Bauer Technique) and Vitek2 as well. Commercially available antibiotic discs were used (Hi Media Laboratories Limited). For isolates of Gram negative Bacilli (ESBL) Extended-spectrum β -lactamase production was screened by testing ceftazidime and cefotaxime, and confirmed with the double-disk diffusion test. MDR organism is defined as non-susceptibility to at least one agent in three or more antimicrobial categories.

RESULT

In our study 259 cases of Fever with Neutropenia were included during September 2014 to September 2016. 189 (73%) patients were from Hematology department. Of these, 55 (21.33%) were found to be culture positive. Highest number of cases were in the young age group. 79 (30.5%) were suffering from Acute Myeloid Leukemia (AML), followed by 73 (28.2%) Acute Lymphocytic Leukemia (ALL).

The occurrence of BSI is inversely proportional to the Absolute Neutrophil count, with highest 15 (88.23%) positive Blood culture was found in patients with ANC < 100/µl (Table 1). 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 also identified. Gram negative bacilli 58.18% (32) is the most common isolate followed by 38.18% (21) Gram positive cocci.

Escherichia coli 50% (16) is the predominant isolate among GNB followed by Klebsiella pneumoniae 43.8% (14) and 6.25% Acinetobacter baumannii complex. 64.3% of Klebsiella pneumoniae and 37.5% of Escherichia coli were found to be Multi Drug Resistant (MDR). 31.3% of the Escherichia coli and 71.4% of Klebsiella were found to ESBL producers.

The isolates were found to be 100% sensitive to only Colistin followed by Teigecycline (93.8%). There is an alarming growth of resistance against commonly used antibiotics and carbepenems (Table 2)

The isolates of Escherichia coli were found to be 100% sensitive to Colistin and Teigecycline. There is an alarming growth of resistance against commonly used antibiotics, carbepenems being only 68.8% sensitive, Ceftrioaxone was found to be 37.5% sensitive (Figure 1)

The isolates of klebsiella pneumoniae were found to be 100% sensitive to Colistin and Teigecycline (85.7%). There is an alarming growth of resistance against commonly used antibiotics and Meropenem, Imipenem being only 64.3% sensitive and Ceftriaxone and piperacillin Tazobactam were 100% resistant (Figure 2). The isolates of Acinetobacter species were found to be sensitive to most of the drugs

DISCUSSION

Patients with malignancies and neutropenia are at higher risk

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-1: Relation of ANC and Culture positive

Isolated GNB		AMP	AMC	PIT	CXM	CTR	CPZ-S	CPM	ERT	IMP	MER	AK	GEN	N	CIP	TGC	COL	COT
Escherichia coli n=16	No.	3	6	11	3	6	11	6	8	11	11	11	14	6	8	16	16	3
	%	18.75	37.5	68.75	18.75	37.5	68.75	37.5	50	68.75	68.75	68.75	87.5	37.5	50	100	100	18.75
klebsiella spp n=14	No.	0	3	0	0	0	3	0	6	9	9	3	3	6	6	12	14	3
	%	0	21.4	0	0	0	21.4	0	42.9	64.3	64.3	21.4	21.4	42.9	42.9	85.7	100	21.4
Acinetobacter spp n= 2	No.	0	0	2	0	1	2	2	2	2	2	2	2	2	2	2	2	2
	%	0	0	100	0	50	100	100	100	100	100	100	100	100	100	100	100	100
Total n= 32	No.	3	9	13	3	7	16	8	16	22	22	16	19	14	16	30	32	8
	%	9.4	28.1	40.6	9.4	21.9	50	25	50	68.8	68.8	50	59.4	43.8	50	93.8	100	25

CIP= Ciprofloxacin, TGC=Teigecycline, COL= Colistin, COT=Cotrimoxazole, AMP=Ampicillin,, 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, AMC= Amoxycillin + clavulinic acid.

Table-1: Antibiotic sensitivity pattern of Gram negative isolates

for the development of Blood stream infection (BSI). The microbiological profile and their sensitivity pattern causing BSI in FN patients have changed over time and geographical location. In the sixties Gram-negative bacteria mainly caused Febrile neutropenia; which gradually changed to Gram-positive bacteria due to increased use of intravascular devices and the introduction of new chemotherapeutic strategies. Gram-negative etiology is now dominant again with emergence of resistant strains that makes the implementation of current strategies for prophylaxis and empirical treatment difficult.[11-13] This warrants a monitoring of the locally prevalent pathogens and their antibiograms periodically, to tailor a rational empiric antimicrobial therapy for neutropenic patients.

In our study, out of 259 cases, 30.5% were cases of Acute Myeloid Leukemia (AML), followed by 28.2% Acute Lymphocytic Leukemia(ALL) with highest number of case 73% were from Haematology department. Similar results reported in other studies, Michelle Karim et al.[14] with 42% cases having AML, followed by 12% ALL. Lakshmaiah C et al.[15] 2, also reported AML as the most common etiology. The high incidence of febrile neutropenia in AML could be explained due to the increased risk of infection due to use of intensive chemotherapy leading to prolonged and profound neutropenia.

In Patients with ANC <100/µl rate of blood culture positivity was found to be highest (87.5%) and only 10.6% in ANC >200/µl confirming that infection is inversely proportional to neutrophil count. According to a study by Bodey et al.[16], the occurrence of infection is highest when absolute neutrophil count falls below100/µl and proportionately less frequent at ANC 100 – 500/ µl and ANC 500-1000/ µl. BSI rate was found to be 21.23% (53) in neutropenic patients. Similar results were reported by –Lakshmaiah C et al.[15], in South India 19.44% positive blood culture in neutropenic patients. But In a study Mandal et al.[17], 2010-2013 in a similar study found 29.1% culture positivity. However in our study no anaerobic organisms were isolated, which is in conformity with other studies Tariq Butt et al.[1] 2002. However Honar Cherif et al.[2] 1988-2001 found 4.1% anaerobic isolates. The positivity rate can be probably explained by the observation made by Matsuhisa et al.[18], that in febrile neutropenic patients most bacteria are phagocytized by neutrophils and only few remain in blood to form colonies detectable by culture.

This study shows the most common isolate is Gram negative bacilli 58.18% (32) followed by 38.18% (21) Gram positive cocci. Other workers in India, Lakshmaiah C et al.[15] 2011 – 2013, reported GNB 57.14% (12), Mandal et al[17], 2010-2013 also reported GNB 61.53% (48) as the predominant isolate. Dong –Gun Lee et al.[19] mentioned GN microorganisms were the major pathogens causing infection, is a general characteristic of Asia-Pacific region.

Escherichia coli 50% (16) is the predominant isolate followed by Klebsiella pneumoniae 43.75% (14) among the 32 Gram negative isolates, which is on a rise and Acinetobacter baumannii complex 6.3% (2). Tariq Butt et al.[1] 2002, reported 13.25% (11) E.Coli as the predominant isolate.

In this study we observed that the GNB isolated were 100% sensitive to Colistin, 93.8% to Teigecycline and only 68.8% sensitive to Imipenem and Meropenem. However contrasting our findings Kuntegowdanahalli C Lakshmaiah et al[15], 2011-2013, found imipenem 100%, Piperacillin-tazoactum 86.95% sensitive. Tariq Butt et al.[1] 2002, also found imipenem to be 100% sensitive.

There is an alarming rise in the MDR GNB isolates with 64.3% of Klebsiella and 37.5% of Escherichia coli being MDR. 31.3% of the Escherichia coli and 71.4% of Klebsiella were found to ESBL producers. In this scenario of increasing resistance to the commonly used antibiotics, this is the need of the hour to tailor an effective empirical therapy based on local and regional prevalence of the causative organisms in neutropenic patients along with their Antibiotic sensitivity pattern for which further investigations in a larger data set is warranted.

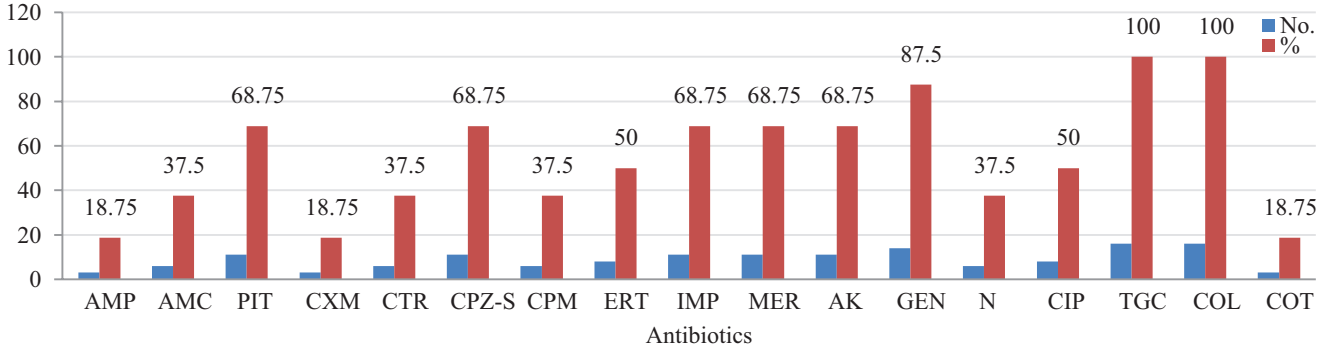

Figure-1: Sensitivity pattern of E.coli

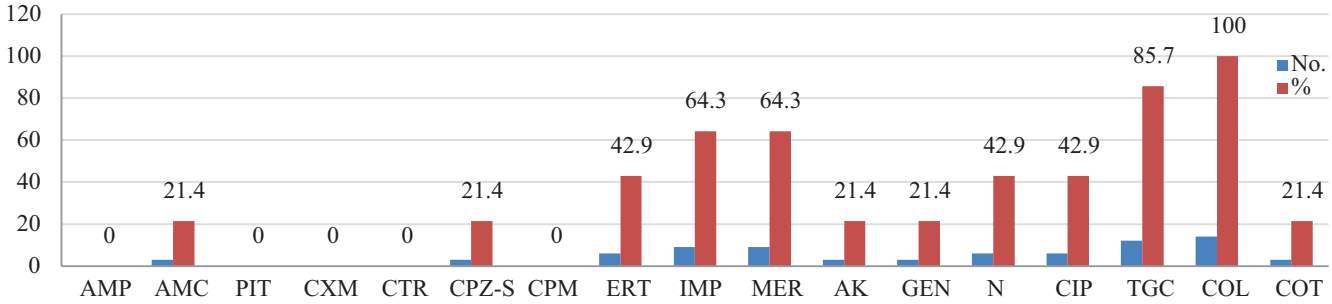

Figure-2: Sensitivity pattern of Klebsiella Species

CONCLUSION

This present study revealed that the frequency of isolation of bacteria in blood in Febrile neutropenic patient is 21.23% and Gram negative bacilli is the predominant pathogens. Gram negative bacilli isolated were highly sensitive to Colistin and Teigecycline, but there is an alarming rise in MDR and ESBL organisms with increasing resistance against carbapenems, cephalosporins and commonly used drugs.

As seen in our study a fall in neutrophil count < 100 makes the individual severely vulnerable to infection by microorganisms, so Blood culture should be done in patients with Febrile Neutropenia and microbiological analysis of these group of patients should be carried out routinely and periodically to compare the changing trends in the microbial aetiology and their antibiogram patterns, so that an effective empirical antibiotic regime could be tailored against the commonly causative organisms.

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