

# Micro-organisms Associated with Febrile Neutropenia in Patients with Haematological Malignancies in a Tertiary Care Hospital in Eastern India

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**Abstract** There is paucity of information from eastern India with regard to observed dominant micro-organisms causing febrile neutropenia (FN) in patients with haematological malignancies. To identify the prevalence of pathogenic microorganisms associated with FN. A total number of 268 episodes of FN were analysed from September'2010 to October'2013. The blood samples were inoculated into brain heart infusion broth, glucose broth, Hicombi dual performance media (Himedia, LQ-12) at 37° C for 168 h and Bactec method was also performed for these samples. Blood agar, chocolate agar, MacConkey's agar and cystine lactose electrolyte deficient agar were used for isolation of the microorganisms. A total number of 78 (29.10 %) episodes revealed positive growths. Gram negative bacilli and Gram positive cocci were isolated in 61.53 and 34.61 % cases respectively. The eight commonest isolates were *Pseudomonas aeruginosa* (14.10 %), methicillin resistant *Staphylococcus aureus* (MRSA-12.82 %), *Acinetobacter* sps (11.53 %), coagulase negative *Staphylococcus* (10.25 %), *Klebsiella pneumoniae* (8.97 %), *Escherichia coli* (8.97 %), ESBL *E. coli* (6.41 %), methicillin sensitive *S. aureus* (MSSA-6.41 %). Amongst other less common isolates were *Citrobacter koseri* (3.84 %), *Citrobacter freundii* (2.56 %), *Ralstonia paucula* (2.56 %), *Cedecia neteri* (1.28 %), methicillin resistant coagulase negative *Staphylococcus* (2.56 %). *Candida* spp. including two cases of *Candida non-*

*albicans* was isolated in 3.84 % of cases. *P. aeruginosa* was the commonest pathogenic isolates in FN patients associated with haematological malignancies in this study. Gram negative bacteria were the commonest isolates in FN including significant numbers of rare opportunistic micro-organisms.

**Keywords** Blood stream infection · Febrile neutropenia · Gram negative organisms

## Introduction

Febrile neutropenia (FN) is considered a medical emergency with a mortality rates of up to 70 % if initiation of antibiotics was delayed [1]. Bacteremia is documented in only 10–25 % of FN episodes and clinically documented infections are found in 20–30 % [2]. Signs and symptoms of infection are often subtle in the neutropenic patient [3] so, the empiric therapy is used to cover the most virulent pathogens that may rapidly cause serious or life-threatening infections. There is paucity of data from this part of the country and this study was conducted to identify the prevalence of pathogenic microorganisms causing infection in FN patients.

## Aims

To identify the prevalence of pathogenic microorganisms associated with FN.

## Materials and Methods

This *prospective study* was conducted at a tertiary hospital for a period of over 3 years from September' 2010 to

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October'2013. Patients admitted in the Hospital with an underlying haematological malignancy and developed FN were included in this study. The medical history including diagnosis, chemotherapy, relapse, duration and severity of neutropenia, granulocyte colony stimulating factor (G-CSF) use and other infection related factors, i.e. antifungal therapy for a probable or proven fungal infection within past 6 months, prophylactic antibiotics or antifungal agents was noted carefully.

Fever was defined as single oral temperature of  $\geq 38.3$  °C or oral temperature of  $\geq 38.0$  °C that persists for over 1 h. Neutropenia was defined as an absolute neutrophil count (ANC) of  $\leq 500$  cells/mm<sup>3</sup>. Bacteraemia was defined as growth of an organism that was judged not to be a contaminant in a blood culture drawn during a febrile episode. Blood cultures (including urine culture, stool culture etc.) were done as per local protocol.

Total of two hundred and sixty eight blood samples were collected from patients of various ages. Blood was collected aseptically from peripheral vein. 10 ml of blood from an adult and 2–5 ml from a young child was collected and equally dispensed into 2 culture bottles, one containing glucose broth, other brain heart infusion broth and same amount of blood was also taken for BACTEC culture. Culture bottles were clearly labeled with the name, number of the patient and the date and time of collection. Culture

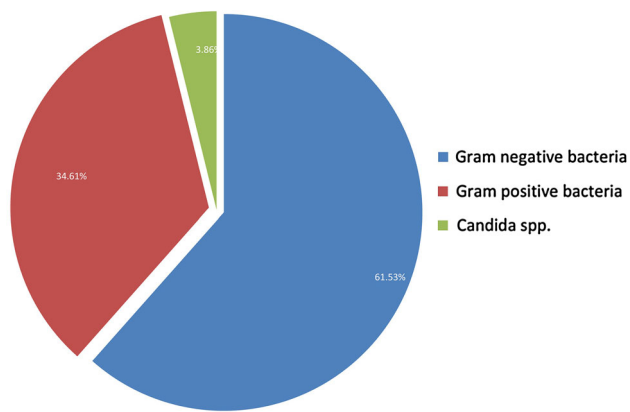
bottles were then incubated at 37 °C for up to 7 days or for a maximum of 14 days anaerobically and aerobically. Bottles were macroscopically examined daily for visible evidence of bacterial growth such as turbidity, haemolysis, gas bubbles and clots. For positive blood samples, sub-cultures were made onto blood agar, chocolate agar and MacConkey agar media. Characteristic colonies thus produced were examined and identified morphologically and biochemically. The organisms were identified through monitoring general biochemical tests such as catalase, oxidase, Triple Sugar Iron agar (TSI), citrate utilization (Simmon's citrates medium), urease (Christensen's Urea Agar), indole, motility, H<sub>2</sub>S production (Sulphide Indole Motility Medium), esculin hydrolysis (Bile esculin agar), and sugar fermentation tests as per standard procedures [4]. All culture Media were provided by Himedia Laboratories Pvt. Ltd., Mumbai; India.

## Results

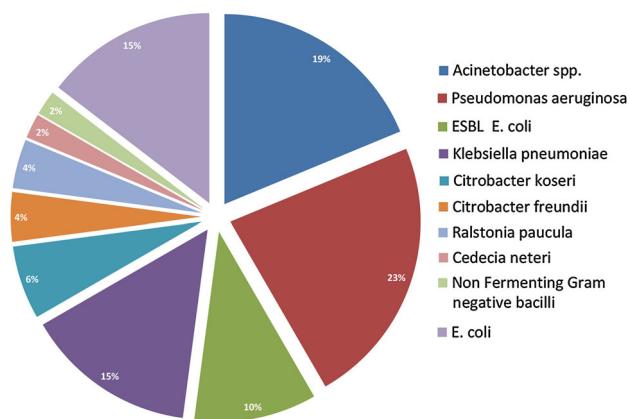
The data analysis of present prospective study revealed that, in a total of 268 blood cultures, 78 yielded microbial growths (Table 1) and in 190 episodes no organism was grown. An incidence of positive blood culture was 29.10 % (78/268) which includes (Fig. 1) Gram negative bacilli

**Table 1** Microorganisms isolated

Sl. No.	Organism	Cases, in respect to total growth positivity (n = 78), No (%)	Cases (%) in respect of total episodes (n = 268)
1.	<i>Pseudomonas aeruginosa</i>	11 (14.10)	4.10
2.	Methicillin resistance <i>Staphylococcus aureus</i> (MRSA)	10 (12.82)	3.73
3.	<i>Acinetobacter spp.</i>	9 (11.53)	3.35
4.	Coagulase negative <i>Staphylococcus</i> (CONS)	8 (10.25)	2.98
5.	<i>Escherichia coli</i>	7 (8.97)	2.61
6.	<i>Klebsiella pneumoniae</i>	7 (8.97)	2.61
7.	Extended Spectrum Beta Lactamase (ESBL) <i>Escherichia coli</i>	5 (6.41)	1.86
8.	Methicillin sensitive <i>Staphylococcus aureus</i> (MSSA)	5 (6.41)	1.86
9.	Methicillin resistance Coagulase negative <i>Staphylococcus</i> (MR-CONS)	2 (2.56)	0.74
10.	<i>Streptococcus pneumoniae</i>	1 (1.28)	0.37
11.	<i>Candida albicans</i>	1 (1.28)	0.37
12.	<i>Citrobacter koseri</i>	3 (3.84)	1.11
13.	<i>Citrobacter freundii</i>	2 (2.56)	0.74
14.	<i>Ralstonia paucula</i>	2 (2.56)	0.74
15.	<i>Cedecia neteri</i>	1 (1.28)	0.37
16.	Non Fermenting Gram negative bacilli	1 (1.28)	0.37
17.	<i>Streptococcus viridians</i>	1 (1.28)	0.37
18.	<i>Candida nonalbicans</i>	2 (2.56)	0.74
	Total positive growth	78	29.10



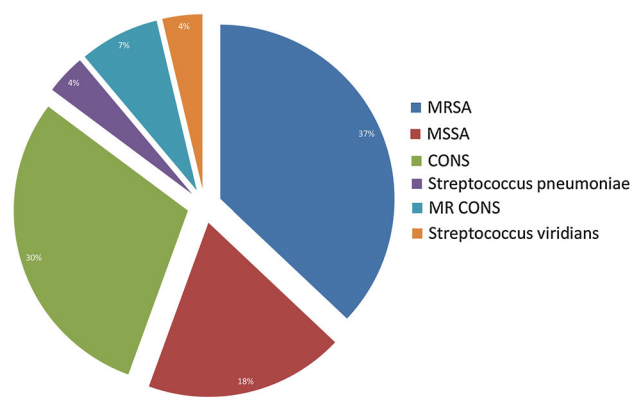
**Fig. 1** Different types of organism isolated



**Fig. 2** Different types of Gram negative organisms isolated. *ESBL* extended spectrum beta lactamase

61.53 % (48/78) and Gram positive cocci 34.61 % (27/78). The common Gram negative organisms (Fig. 2) isolated in this group are *Pseudomonas aeruginosa* (14.10 %), *Klebsiella* spp. (8.97 %) and *Escherichia coli* (8.97 %). *Acinetobacter* spp. was isolated in nine (11.53 %) cases, defined in the group of Enterobacteriaceae. *Citrobacter koseri* (3.84 %), *Citrobacter freundii* (2.56 %), *Cedecia neteri* (1.28 %) are the remote bacteria from the pragmatic patients. Some unusual bacteria like *Ralstonia paucula* was also found in 2.56 % of gram negative isolates.

Gram-positive bacteria (Fig. 3) accounted for one third of microbiologically documented infections. Methicillin resistance *Staphylococcus aureus* (MRSA-12.82 %) and coagulase negative *Staphylococcus* spp. (CONS-10.25 %) were the main Gram positive pathogens. Of the 27 Gram positive isolates, *S. aureus* susceptible to methicillin was detected in 6.41 % cases. *Candida* spp. (both *C. albicans* and *C. nonalbicans*) were present in three (3.86 %) cases.



**Fig. 3** Different types of Gram positive organisms isolated. *MRSA* methicillin resistance *Staphylococcus aureus*, *MSSA* methicillin sensitive *Staphylococcus aureus*, *CONS* coagulase negative *Staphylococcus*, *MR CONS* methicillin resistance coagulase negative *Staphylococcus*

## Discussion

Blood stream infections (BSIs) are a cause of significant morbidity and mortality in neutropenic patients. The causative organisms of BSIs have changed over time. In the 1960s to the 1970s, Gram negative bacteria were more predominant causative agents, but over the last few decades there has been a shift toward Gram positive bacteria due to routine use of anti microbial prophylaxis. Our study however revealed that Gram negative bacteria are predominant isolates from FN patients. Gram-negative bacterial infections represent two third of the microbiologically documented infections in most febrile series of neutropenic patients [5–9]. The high occurrence of non-lactose fermenters especially *Pseudomonas* spp. and *Acinetobacter* spp. was a matter of concern. Both of these are associated with a high degree of resistance to antibiotics. It was documented that infections with *P. aeruginosa* have been associated with increased mortality. The most frequently identified species were *P. aeruginosa* in this study. *P. aeruginosa* was isolated in 50 % of neutropenia cases with overall mortality around 10 % in other studies [6, 7, 10–12]. In a study [13] from All India Institute of Medical Sciences, New Delhi, India analyzing for BSIs, pathogen profiles and antimicrobial resistance in FN patients with haematological cancers and bone marrow failure syndromes showed *Acinetobacter* spp. emerging as common pathogen. *Acinetobacter* and *pseudomonas* together were responsible for 42.9 % of all BSIs and both displayed very high resistance to all major classes of antibiotics, including multidrug resistance and ESBL production.

$\beta$ -lactamases are one of the most important mechanisms of resistance in Gram negative bacteria. Based on this finding, several studies have recommended for first-line empiric antibiotic therapy in gram negative infections [6,

10–12, 14, 15]. Extended Spectrum Beta Lactamase (ESBL) *E.coli* was encountered in 6.41 % of the total growths in our study. *Acinetobacter* spp. and *Citrobacter* spp. have emerged as prominent multi drug resistant (MDR) bacteria and their occurrence in the setting of malignancy could be disastrous. *R. paucula* and *C. neteri* were grown in few patients and these opportunistic organisms are very rarely reported in FN cases. *Use of gram negative prophylaxis (e.g. ciprofloxacin and levofloxacin) for neutropenic fever has caused the emergence of gram positive organisms as dominant infective pathogens.* Among patients with FN, 80 % of gram positive bacteraemia were caused by CONS and viridians Streptococci. CONS are the most common cause of infection in majority of studies [16–19]. Staphylococcal bacteremia results in a low mortality rate, whereas oral streptococci, which are responsible for up to 39 % of infections in neutropenic hosts [20], have been associated with mortality rates of 4–22 % [21, 22]. In a recent study [23] from India retrospectively analyzing the clinical profile and bacterial spectrum and sensitivity patterns of pathogens in febrile neutropenic patients in hematological malignancies revealed gram negative organisms are the predominant organisms with *E. coli* as major isolate while *S. aureus* representing the most common gram positive organism.

In addition to gram-negative and gram positive bacteraemias, *Candida* spp. (both *C. albicans* and non-*albicans Candida* spp.) were also found in our study. Invasive fungal infection is becoming increasingly important in critically ill patients. *Candida* has become the fourth most common organism responsible for BSIs in the intensive care unit (ICU) [24, 25].

*In conclusion*, in approximately one third of cases of FN, causative organism could be identified. Gram negative followed by gram positive organisms and *Candida* spp. are common isolated pathogens. Isolation of MDR bacteria (*Acinetobacter* spp. and *Citrobacter* spp.) and growth of unusual organism (*R. paucula* and *C. neteri*) are important findings in this study. *Therefore, blood cultures and sensitivity pattern in the locality are essential for surveillance purposes to guide clinicians on proper management of FN patients.*

**Conflict of interest** The authors of this paper have no conflicts of interest.

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