www.jmscr.igmpublication.org
Index Copernicus Value: 79.54
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v7i6.177

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Original Article

Antimicrobial Susceptibility Pattern of the Blood Stream Infection Isolates in Neutropenic Patients on Chemotherapy for Solid Malignancies

Author
Dr Yazhini K, MBBS., MD.
Department of Microbiology, Government Medical College, Thrissur, Kerala, India
Corresponding Author
Dr Yazhini K, MBBS., MD
Assistant Professor & Consultant Microbiolgist, Meenakshi Ammal Dental College & Meenakshi General Hospital
Address: No.12, Padmavathy nagar, 13th cross street, Thirumullaivoyal, Chennai – 600062, India

Abstract

Background: *Empiric broad-spectrum antibiotic therapy has been the cornerstone of the management of fever in patients with chemotherapy-induced neutropenia. In the face of emerging multidrug-resistant organisms, antimicrobial prophylaxis and treatment have become increasingly difficult in these highly compromised patients. Thus, the local data of common pathogens is important to initiate the appropriate empirical antibiotic therapy.*

Objectives: *To determine the antimicrobial susceptibility pattern of the blood stream infection isolates in neutropenic patients receiving chemotherapy for solid tumours.*

Methods: *Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per Clinical Laboratory Standards Institute (CLSI) guidelines. Antibiotic discs were procured from Himedia, Mumbai. The diameters of zones of inhibition were interpreted according to CLSI standards for each organism. Culture media and antibiotic discs were tested for quality control using standard ATCC strains.*

Result: *Antimicrobial susceptibility testing was done as per CLSI guidelines. The Gram positive isolates showed 100% susceptibility to Vancomycin. Methicillin resistance was seen among 24% of Staphylococcus epidermidis isolates. Staphylococcus aureus isolates were uniformly susceptible to Methicillin. All the Gram negative isolates were found to be ESBL producers by ESBL phenotypic confirmatory double disc test.*

Conclusion: *The findings highlight the role of Staphylococcus epidermidis, which is commonly considered as a commensal, as a potential pathogen in neutropenic patients, and necessitate the inclusion of antimicrobials having gram positive coverage in the empirical antimicrobial therapy.*

Keywords: *Neutropenia, Chemotherapy, Blood stream infection, Solid malignancy, Antimicrobial susceptibility.*

Introduction

The increasing use of cytotoxic chemotherapy in patients with solid tumors has increased the number of patients who have neutropenia.[1] Neutropenic patients are vulnerable to a wide spectrum of infectious agents which cause

substantial mortality and morbidity among them. In addition, neutropenia blunts the inflammatory response to nascent infections, allowing bacterial multiplication and invasion.[2] Bloodstream infection (BSI) by far is the most common complication in patients with cancer leading to delayed and reduced dosage of chemotherapeutics and longer hospitalization.[3-5]

The relationship between infections and neutropenia was first described as early as 1960s in patients with acute leukemia receiving chemotherapy.[6] Since then, considerable progress has been made in the management of fever and infection in neutropenic patients. Empiric broad-spectrum antibiotic therapy has been the cornerstone of the management of fever in patients with chemotherapy-induced neutropenia.[1] In the face of emerging multidrug-resistant organisms, antimicrobial prophylaxis and treatment have become increasingly difficult in these highly compromised patients.

Patients with solid tumours are a unique cohort; they frequently have implantable devices and are relatively immunocompromised, even without overt neutropenia. Surprisingly, only limited data have been reported on BSI in patients with solid tumors, in terms of the current epidemiology, etiology, impact of MDR organisms, and outcomes.[7,8] Furthermore, because of higher morbidity and mortality of infection in these patients, antibiotic therapy should be started as soon as possible. Thus, the local data of common pathogens is important to initiate the appropriate empirical antibiotic therapy. This study was conducted to assess the antimicrobial susceptibility pattern of BSI isolates in patients with neutropenia receiving chemotherapy for solid tumours.

Materials and Methods

Study design: Cross sectional study.

Sample size: 150

Study population

Adult patients between 18 to 65 years of age, who received chemotherapy for solid malignancies, admitted with neutropenia to the Oncology ward in Government Medical College, Thrissur.

Ethical consideration

Approval was obtained from the institutional ethical committee before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

Budget of the study

The sampling and processing was be done by the investigator. Study did not cause any additional financial burden on the patient.

Study Procedure

Identification of the isolates

The isolates were identified based on colony morphology,[9] Gram staining, motility, VITEK 2 identification systems and biochemical reactions by standard microbiological techniques.

The control organisms *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC BAA-747, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 were used to check the quality of media and reagents and to evaluate colour stability.

Antimicrobial Sensitivity Testing

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per Clinical Laboratory Standards Institute (CLSI) guidelines. Antibiotic discs were procured from Himedia, Mumbai. The diameters of zones of inhibition were interpreted according to CLSI standards for each organism.[10] Culture media and antibiotic discs were tested for quality control using standard ATCC strains.

The following standard strains were used for quality control:

Staplylococcus aureus – ATCC 25923

Escherichia coli – ATCC 25922

Pseudomonas aeruginosa – ATCC 27853

Table 1 Antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method as per CLSI guidelines

Medium	Mueller-Hinton Agar (MHA)
Inoculum	0.5 McFarland Standard
Incubation	16-18 hrs / 37˚C

Table 2 Panel of antimicrobials tested for Gram Negative bacteria[11]

ANTIMICROBIAL AGENT	DISC CONTENT	ZONE DIAMETER (mm)			
		S	SDD	I	R
Ampicillin	10 µg	≥17	-	14-16	≤13
Amikacin	30 µg	≥17	-	15-16	≤14
Gentamicin	10 µg	≥15	-	13-14	≤12
Ceftriaxone	30 µg	≥23	-	20-22	≤19
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	-	11-15	≤10
Ciprofloxacin	5 µg	≥21	-	16-20	≤5
Ceftazidime	30 µg	≥21	-	18-20	≤17
Cefepime	30 µg	≥25	19-24	-	≤18
Piperacillin-Tazobactam	100/10 µg	≥21	-	18-20	≤17
Imipenem	10 µg	≥23		20-22	≤19

Table 3 Panel of antimicrobials tested for *Staphylococcus spp.*

ANTIMICROBIAL AGENT	DISC CONTENT	ZONE DIAMETER (mm)		
		S	I	R
Penicillin	10 units	≥29	-	≤28
Erythromycin	15 µg	≥23	14-22	≤13
Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤10
Cefoxitin (for *S. aureus*&*S. lugdunensis*)	30 µg	≥22	-	≤21
Cefoxitin (for CoNS except *S. lugdunensis*)	30 µg	≥25	-	≤24

Table 4 Panel of antimicrobials tested for *Enterococcus spp.*

ANTIMICROBIAL AGENT	DISC CONTENT	ZONE DIAMETER (mm)		
		S	I	R
Penicillin	10 units	≥15	-	≤14
Ampicillin	10 µg	≥17	-	≤16
Vancomycin	30 µg	≥17	15-16	≤14

Detection of Extended Spectrum Beta Lactamase Production in Gram Negative Bacteria

Screening method: In keeping with the Clinical and Laboratory Standards Institute (CLSI) recommended guidelines, ESBL screening was performed by disk diffusion using Ceftazidime (30 µg) disc. Isolates of gram negative bacilli showing resistance to any of the indicator Cephalosporins given below were considered to be possible ESBL producing strains and subjected to confirmatory test.[12]

Table 5 Screening for ESBL producers

ANTIMICROBIAL DISC & CONTENT	ZONE DIAMETER FOR POSSIBLE ESBL PRODUCING STRAIN
Cefotaxime (30µg)	≤22 mm
Ceftazidime (30 µg)	≤17 mm
Ceftriaxone (30 µg)	≤19 mm

Confirmatory tests: Confirmation of ESBL phenotype was performed by the double disk diffusion method using antibiotic discs containing a combination of Cephalosporin plus Clavulanic acid in conjunction with a corresponding cephalosporin disk alone. The following antibiotic disks were used: Ceftazidime (CAZ 30µg), Ceftazidime plus Clavulanic acid (CAZ/CA 30/10µg).[12]

Detection of AMPC B-Lactamase Production in Gram Negative Bacteria

Screening method: A 0.5 McFarland of test isolate was swabbed on Mueller Hinton Agar plates and discs of Cefotaxime (30 µg) and/or Ceftazidime (30 µg) were placed adjacent to Cefoxitin (30 µg) disc at a distance of 20 mm from each other. Isolates showing blunting of Ceftazidime or Cefotaxime zone of inhibition

adjacent to Cefoxitin disc or showing reduced susceptibility to either of the above test drugs (Ceftazidime or Cefotaxime) and Cefoxitin were considered as "screen positive" and selected for detection of AmpC β-lactamases.

AmpC disc test

A lawn culture of *E. coli* ATCC 25922 was prepared on MHA plate. Sterile discs (6 mm) were moistened with sterile saline (20 µl) and inoculated with several colonies of test organism. The inoculated disc was then placed beside a Cefoxitin disc (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C. A positive test appeared as a flattening or indentation of the Cefoxitin inhibition zone in the vicinity of the test disk. A negative test had an undistorted zone.[13,14]

Detection of Methicillin Resistance in Staphylococcus Aureus

Disc diffusion method: Colonies isolated from agar culture plate were suspended directly into broth, vortexed to reach 0.5 Mc Farland's standard. A lawn culture of the staphylococcal colonies was made on the MHA plate and Cefoxitin (CX 30µg) disc was applied and incubated at 35˚C for 24 hours. According to CLSI criteria, a diameter of ≤21 or ≥22 mm correspond to resistant or susceptible to Cefoxitin respectively.[15]

Detection of Vancomycin MIC for *Staphylococcus aureus* isolates by Epsilometer test (E test): 0.5 McFarland suspension of 24 hour old test isolate, grown on a non specific medium, was prepared and lawn cultured onto Mueller Hinton agar. E strip of Vancomycin-Cefoxitin Ezy MIC strip (HiMedia) was placed on the surface of agar and the plates were incubated at 35˚C for 18-24 hrs and interpreted for MIC detection. MIC of the drug was taken at the point where the ellipse intersects the MIC scale on the strip. Control strain ATCC *Staphylococcus aureus* 25923 were tested in parallels.[13]

Interpretation criteria

For Vancomycin MIC values: MIC < 2µg/ml – Sensitive

MIC 4-8 µg/ml – Intermediate

MIC >16 µg/ml – Resistant

For CefoxitinMIC values: MIC > 6 µg/ml – MRSA strain

MIC ≤ 6 µg/ml – MSSA strain

Results

Table 6: Distribution of Microbiologically documented BSI among patients with CIN

Microbiologically documented BSI	No. of patients	Percentage
Present	30	20%
Absent	120	80%

Among the patients with CIN, 20% (n=30) of the patients had Microbiologically documented BSI.

Among the total isolates, *Staphylococcus epidermidis* was the predominant isolate which comprised 83.3% (n=25), followed by *Staphylococcus aureus* that comprised 6.6% (n=2).

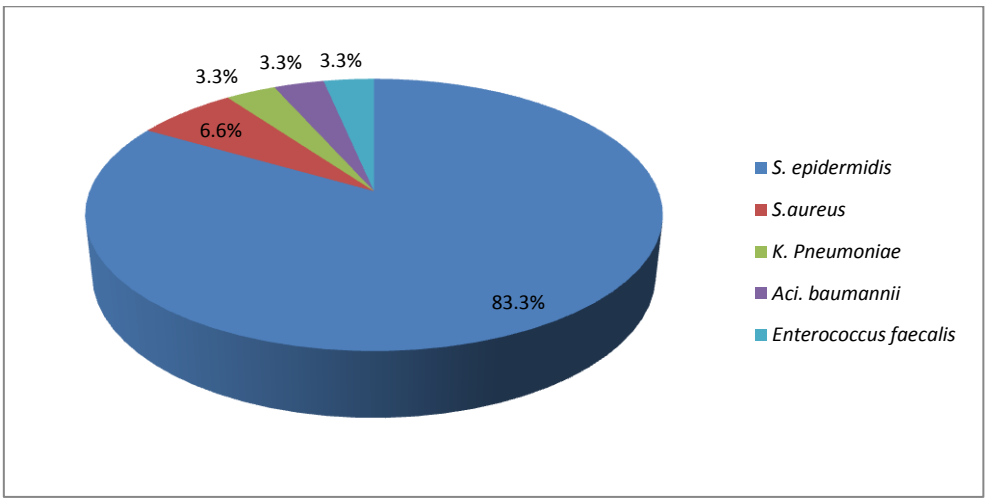

Figure 1: Comparative predominance etiological agents causing BSI in patients with CIN

Table 7: Susceptibility of *Staphyloccus spp.* to Cefoxitin

Organism	Susceptible (%)	Resistant
S. epidermidis	19 (76%)	6 (24%)
S. aureus	2 (100%)	0 (0%)

Among the *Staphylococcus epidermidis* isolates, 76% (n=19) were susceptible to Cefoxitin which is a surrogate marker for Methicillin, and 24% (n=6) showed resistance to Cefoxitin.

Staphylococcus aureus isolates showed 100% susceptibility to Cefoxitin. Hence the prevalence of MRSA in this study counts to zero.

Table 8: Distribution of Gram positive isolates susceptible to Vancomycin (n=28)

Organism	Susceptible	Resistant
S. epidermidis	25	0
S. aureus	2	0
E. faecalis	1	0

Among the Gram positive isolates, *Staphylococcus epidermidis, Staphylococcus aureus and Enterococcus faecalis* showed 100% susceptibility to Vancomycin.

Table 9: Distribution of Gram negative isolates producing ESBL (n=2)

Organism	ESBL producer	Not an ESBL producer
Acinetobacter baumannii	1	0
Klebsiella pneumoniae	1	0

Among the Gram negative isolates, one *Klebsiella pneumoniae* and one *Acinetobacter baumannii* isolated were found to be ESBL producers, which accounts to 100%.

Among the *Staphylococcus* isolates, 93% (n=25) were resistant to Penicillin and only 7% (n=2) were susceptible to Penicillin, and 78% (n=21) were susceptible to Cefoxitin, and 22% (n=6) showed resistance to Cefoxitin.

All the *Staphylococcus* isolates showed 100% susceptibility to Vancomycin.

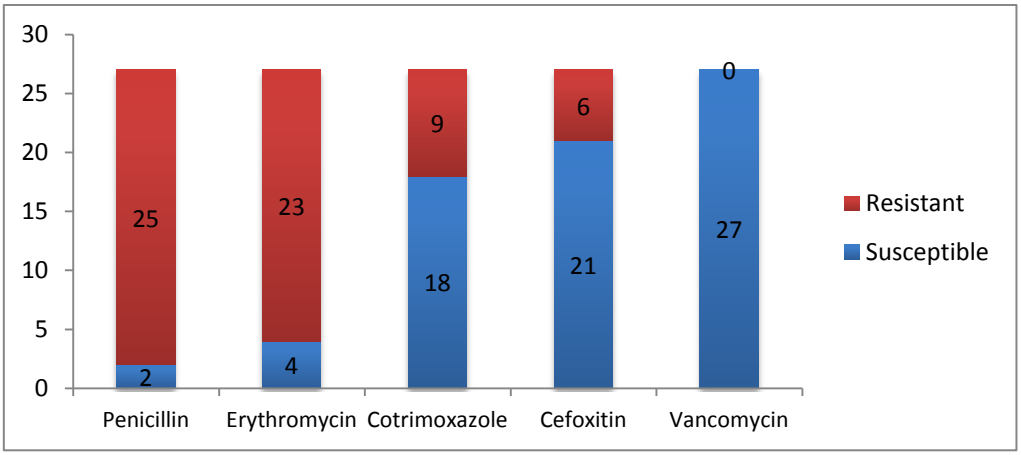

Figure 2: Antimicrobial Susceptibility pattern of *Staphylococcus* isolates

The *Enterococcus faecalis* isolate was resistant to Penicillin and Ampicillin and was Susceptible to Vancomycin.

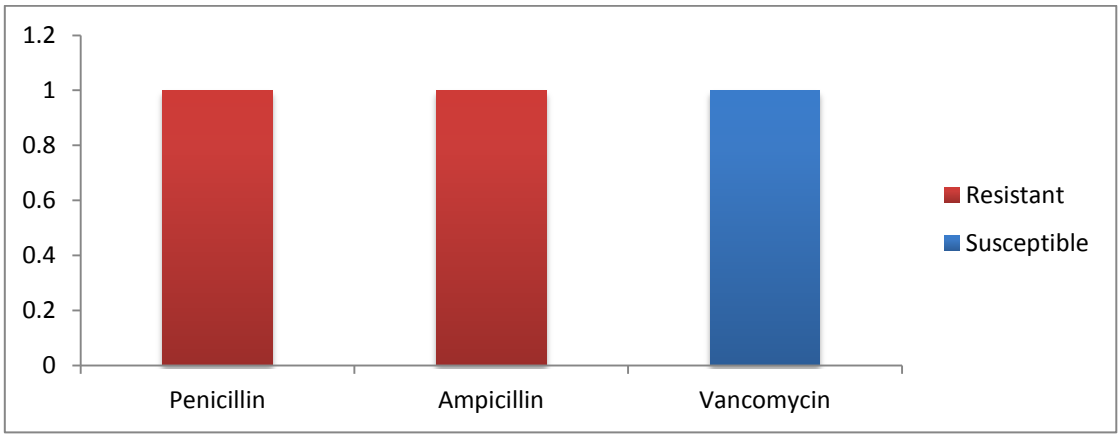

Figure 3: Antimicrobial Susceptibility pattern of *Enterococcus faecalis*

The *Acinetobacter baumannii* isolated was found to be susceptible to Cotrimoxazole, Ciprofloxacin, Amikacin, Cefipime, Piperacillin + Tazobactam and Imipenam, and was resistant to Ceftriaxone. The isolate was also found to be an ESBL producer.

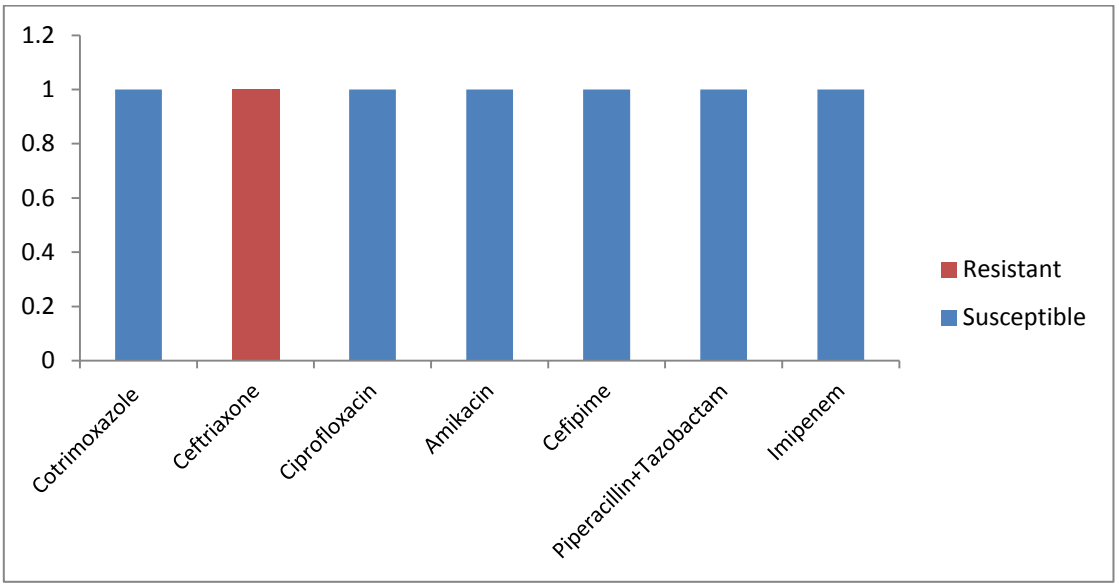

Figure 4: Antimicrobial Susceptibility pattern of *Acinetobacter baumannii*

The Klebsiella pneumonia isolate was found to be susceptible to Ciprofloxacin, Amikacin, Cefipime, Piperacillin + Tazobactam and Imipenem, and was resistant to Cotrimoxazole and Ceftrixone. The isolate was also found to be an ESBL producer.

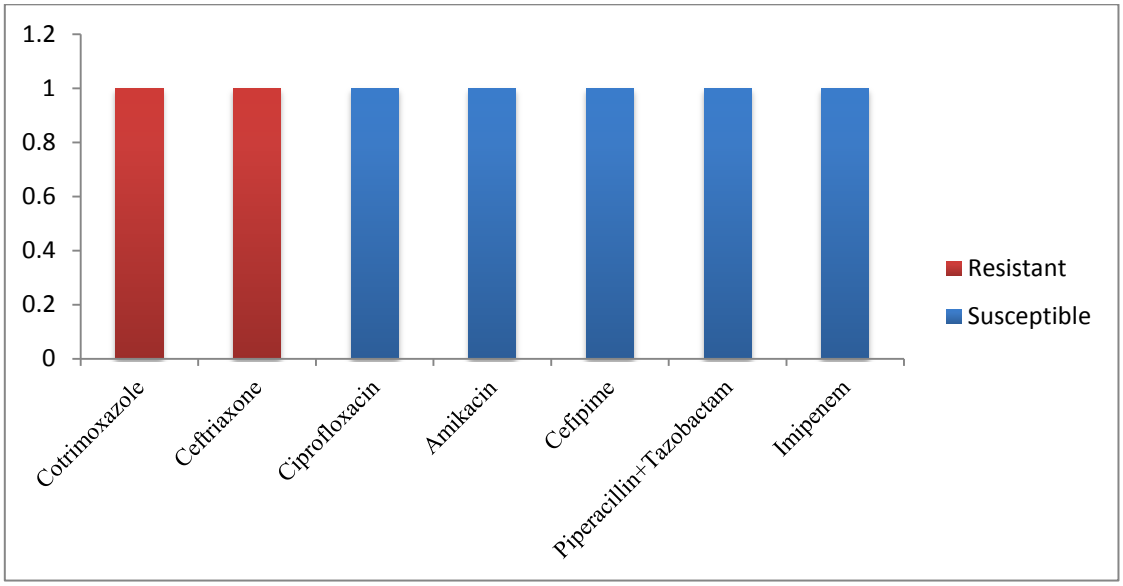

Figure 5: Antimicrobial Susceptibility pattern of *Klebsiella pneumonia*

Discussion

The proportion of BSIs caused by gram-positive organisms has increased considerably in recent years, and figures of 70%–81% have been reported.[16,17] In this study, among the 30 cases of microbiologically documented BSI, Gram positive cocci contributed to 89% (n=28) and Gram negative bacilli 11% (n=2). This corresponds to the study by Gonzalez-Barca E *et al*[16] that reported Gram positive bacteremia in 81% among the isolates and Rubio M *et al*[17] that reported Gram positive bacteremia to be 70%. But this is in

contrast to the study conducted by Prabhash K *et al.* (2010)[18] at Tata Memorial Hospital, Mumbai, Maharashtra, India, where Gram-negative bacteria were more common as etiologic agents of BSIs in cancer patients.

Patients 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.

Among the Gram positive cocci (n=28) causing BSI, *Staphylococcus epidermidis* was the predominant isolate comprising 89.29% (n=25). *Staphylococcus aureus* and *Enterococcus faecalis* comprised 7.14% (n=2) and 3.57% (n=1) respectively.

According to The Infectious Diseases Society of America (IDSA) 2010 update, coagulase-negative staphylococci are the most common blood isolates in most centers.[3]A large longitudinal study performed in the United Kingdom[19]comparing the etiology of BSI in hematologic and oncology patients also found a predominance of gram-positive organisms, with coagulase-negative staphylococci being the most frequent bacteria causing BSI in both patient groups. Confirming these studies, we also found that gram-positive organisms are the most prevalent pathogens causing BSIs in patients with solid malignancies.

Antimicrobial susceptibility testing was done as per CLSI guidelines. Among the Gram positive isolates, *Staphylococcus epidermidis, Staphylococcus aureus and Enterococcus faecalis* showed 100% susceptibility to Vancomycin and were well within the sensitive range.

Methicillin resistance was seen among 24% of *Staphylococcus epidermidis* isolates. *Staphylococcus aureus* isolates were uniformly susceptible to Methicillin.

Among the Gram negative isolates, one *Klebsiella pneumoniae* and one *Acinetobacter baumannii* isolated were found to be ESBL producers by ESBL phenotypic confirmatory double disc test. But since the overall Gram negative isolates were less (n=2), the prevalence of ESBL producers among neutropenic patients, which accounts to 100% in this study, could not be commented on. ESBL bacteraemia is frequent among cancer patients, especially in those exposed to antibiotic pressure.[20]We observed that both the patients harbouring an ESBL strain received an initial empirical antimicrobial therapy.

Conclusion

Among the documented cases of blood stream infections, Gram positive cocci contributed to 89 percent and Gram negative bacilli contributed to 11 percent of total isolates. Among the Gram positive cocci, *Staphylococcus epidermidis* was the predominant isolate. The Gram positive isolates showed 100% susceptibility to Vancomycin. Methicillin resistance was observed in 24% of *Staphylococcus epidermidis* isolates. *Staphylococcus aureus* isolates were uniformly susceptible to Methicillin. On the other hand, all the Gram negative isolates were found to be ESBL producers. The findings highlight the role of *Staphylococcus epidermidis*, which is commonly considered as a commensal, as a potential pathogen in neutropenic patients, and necessitate the inclusion of antimicrobials having gram positive coverage in the empirical antimicrobial therapy.

Declaration of Interest: None

References

1. Pizzo PA. Management of Fever in Patients with Cancer and Treatment-induced Neutropenia. N Engl J Med. 1993;328:1323–32.
2. National Cancer Institute. Common toxicity criteria, version 2.0. Available from URL: http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf [access date January 3, 2003].
3. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, Raad II, Rolston KV, Young JA, Wingard JR.

Infectious Diseases Society of America (2011) clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2011; 52: 427–431.

4. Klastersky J. Management of fever in neutropenic patients with different risks of complications. *Clin Infect Dis*. 2004; 39: S327.
5. Montassier E, Batard E, Gastinne T, Potel G, de la Cochetiere MF. Recent changes in bacteraemia in patients with cancer: a systematic review of epidemiology and antibiotic resistance. *Eur J Clin Microbiol Infect Dis*. 2013; 32: 841–850.
6. Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia *Ann Intern Med* 1966; 64: 328-39.
7. Anatoliotaki M, Valatas V, Mantadakis E, Apostolakou H, Mavroudis D, Georgoulias V, Rolston KV, Kontoyiannis DP, Galanakis E, Samonis G. Bloodstream infections in patients with solid tumors: associated factors, microbial spectrum and outcome. *Infection*. 2004; 32: 65–71.
8. Schelenz S, Nwaka D, Hunter PR. Longitudinal surveillance of bacteraemia in haematology and oncology patients at a UK cancer centre and the impact of ciprofloxacin use on antimicrobial resistance. *J Antimicrob Chemother*. 2013; 68: 1431–1438.
9. Betty A.Forbes, Daniel. F. Sahm, Alice S. Weissfeld *Bailey & Scott's Diagnostic Microbiology* 12th edition.
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved standard, 9 ed. Document M2-A9. Wayne, PA: CLSI; 2006.
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. M100-S25.Table 2A,2B-1,2B-2.Jan 2015. Vol.35No.3.
12. Screening and confirmatory test for ESBL's in Enterobacteriaceae isolates. CLSI document M100-S25.Table 3A.Jan 2015.Vol.35No.3.
13. Workshop manual – Antimicrobial susceptibility testing methods. Dept of Microbiology, JSS Medical College and Hospital, Mysore,India.
14. Singhal et al. Evaluation of methods for AmpC beta lactamases in gram negative isolates from tertiary care hospitals. Indian journal medical microbiology 2005 23(2)120-124.
15. Screening test for detection of Methicillin Resistance (Oxacillin Resistance) in *Staphylococcus* species. CLSI document M100-S25.Table 3E.Jan 2015.Vol.35No.3.
16. Gonzalez-Barca E, Fernandez-Sevilla A, Carratala J, Granena A, Gudiol F. Prospective study of 288 episodes of bacteremia in neutropenic cancer patients in a single institution. Eur J Clin Microbiol Infect Dis 1996;15:291-6.
17. Rubio M, Palau L, Vivas JR, et al. Predominance of gram-positive microorganisms as a cause of septicemia in patients with hematological malignancies. Infect Control Hosp Epidemiol 1994;15:101-4.
18. Prabhash, K., Medhekar, A., Ghadyalpatil, N., Noronha, V., Biswas, S., Kurkure, P., Nair, R. and Kelkar, R. (2010), "Blood stream infections in cancer patients: A single center experience of isolates and sensitivity pattern." *Indian Journal of Cancer*, 47(2), 184-88.
19. Mandal, P.K, Maji, S.K., Dolai, T.K., De, R., Dutta, S., Saha, S. and Bhattacharyya, M. (2015). "Micro-organisms Associated with Febrile Neutropenia in Patients with

Haematological Malignancies in a Tertiary Care Hospital in Eastern India." *Indian Journal of Hematology and Blood Transfusion*,31(1), 46-50.

20. Gudiol, C., Calatayud, L., Garcia-Vidal, C., Lora-Tamayo, J., Cisnal, M., Duarte, R., Arnan, M., Marin, M., Gudiol, F. and Carratala, J. (2010). "Bacteraemia due to extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) in cancer patients: clinical features, risk factors, molecular epidemiology and outcome." *Journal of Antimicrobial Chemotherapy*, 65(2), 333-41.