Blood Stream Infections in Immunocompromised Cancer Patients Detected by Automated Blood Culture System - A Retrospective Analysis

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Summary

For the diagnosis of bacteremias/ fungemia's automated blood culture system like BACTEC 9050 / FX40 is helpful in quick identification of the causative agent and further help treatment of these critically ill patients. Aim was to retrospectively analyze the growth of the pathogenic organisms causing blood stream infections in immunocompromised cancer patients and performing the antibiotic sensitivity testing using automated Identification(ID) and antibiotic sensitivity testing(AST). Standard bacteriological methods were followed for the diagnosis of etiological agent. Automated blood culture system flagged positive and negative cultures. Further the isolated organisms were followed up for the identification and susceptibility testing using automated Vitek-2 Compact. The results were entered in WHO Net system and data was analyzed. Out of the total 1590 blood cultures received, 18.3%were blood culture positive. Growth of Gram-Negative bacilli outnumbered than the gram-positive cocci. Out of the non – lactose fermenting bacilli (NLF), Acinetobacter baumanii showed 20-100% resistance to different antibiotics tested. Burkohlderia showed 19.6-100% resistance to different antibiotics whereas pseudomonas sp. showed around 50 % resistance to all antibiotics. Amongst the Lactose fermenter , E. Coli showed 33.3-100% resistance to most of the antibiotics. The antibiotic sensitivity of gram-positive cocci showed that 0% of Enterococcus faecalis were resistant to Vancomycin, 75% of Enterococcus faecium were resistant to vancomycin. Other gram-positive Cocci showed variable resistance to the panel of different antibiotics. Almost all species of staphylococci are beta Lactamase producers and were resistant to penicillin and cephalosporins. S. *capitis* showed 100% resistance to most of the antibiotics, and Vancomycin resistance was also seen in S. Xyloses (100%), S. epidermidis.(15.4%) and S. aureus (8.3%). It was also observed that Candidemia was caused by C. albicans and glabrata. In Conclusion, we can say that gram negative bacilli are the predominantly isolated bacteria which caused mortality, complications and late recovery from the disease due to antibiotic resistance. The automation in microbiology helped in early reporting and thus early institution of antibiotics to the patients.

Keywords: Immunocompromised Cancer Patients, Automated blood Culture system, GPC, GNB.

Introduction

Blood stream infections are threatening and cautionary leading to morbidity and mortality in cancer patient. It is very crucial and important to detect Bacteremia and Fungemia on an urgent basis and use methods that rapidly detect the presence of clinically important bacteria/Fungi.[1]

Earlier conventional technique was used which comprised of biphasic media to detect the growth of aerobic bacteria. After inoculation of blood, the broth is tilted on the slant twice on the first two days and once daily until day seven. They were tedious and time consuming. Whereas automated methods to detect bacterial growth from patients suffering with bacteremias is by detecting liberated CO_2 by fluorescence at the bottom of the bottles.[2] The aim of this retrospective analysis of bacteremia / fungemia is to generate a meaningful data to know the trends of the causative agents and their antibiotic/ antifungal resistance and to design antibiotic policy.

Materials and Methods

The data analysis was done from November 2018 to April 2019 (a period of 6 month) in the Microbiology laboratory of the Gujarat Cancer Research Institute, Ahmedabad. The subjects were patients suffering with different types of cancers and they had signs and symptoms of blood stream infections like persistent fever, chills, rigors and other systemic symptoms in alignment with septicemia.

As per routine laboratory set up, patients 8-10 ml of blood from adult and pediatric patient was collected aseptically in blood culture (BC) bottle from Becton Dickinson. The bottles were incubated for five days until negative as per set protocol. Total number of days was noted for positive culture bottle. Follow up from the positive culture bottle was done as per the laboratory policy. The organisms causing bacteremia were identified and further processed for antibiotics susceptibility testing by automated ID and AST system (Vitek-2 compact).The results were noted and dispatched through WHONET and laboratory information system (LIS).

Results

Automated blood culture system really helped in hastening up the yield of bacteria / fungi as an early and rapid diagnosis. Table-1 shows that the number of days of incubation of blood culture bottles was done in the automated BC system. It was observed that the detection of bacteria /fungus was in one day (24 hours) which accounted to 30.66 average events. Blood stream infections were 27.90% (1590/5697) of total culture/sensitivity samples received in the laboratory. They were requested by the clinicians in those patients

Table 1 : Blood culture positive by automated BACTEC automated system

Sr. No.	Positive cultures in days	Events Positive -2018 (6 Months)						
		November	December	January	February	March	April	Average events
1	One day	27	22	26	25	48	36	30.66
2	Two days	11	14	12	07	17	14	12.5
3	Three days	09	01	02	02	03	01	3.00
4	Four days	05	01	02	00	01	04	2.16
5	Five Days	02	00	02	06	00	03	1.16

Table 2: Blood Cultures requested in patients suffering with different Cancer (n=1591)

Diagnosis	Number of Blood cultures	(%)
CNS cancer	0001	0.06
GI cancer	0044	2.76
Gynec cancer	0035	2.19
Head & Neck cancer	0037	2.32
Leukemia	1027	64.5
Lymphoma	0046	2.89
Musculoskeletal	0004	0.25
Other tumor	0377	23.6
Respiratory cancer	0019	1.19

who were critical and were suffering with continuous fever and not relieved by empiric antibiotic therapy. Table-2 shows the clinical diagnosis of the patients whose blood cultures were received. 64.5% (1027/1591) of leukemia patients were critically ill and were immune compromised. Out of 1591 blood cultures, 20.6% (329/1591) were categorized as others which included breast cancer, neonatal mass, perineal mass, pancytopenia, pelvic mass, glioma, teratoma, meningioma etc. It was observed that the blood cultures from adults (862) were more than paediatric (729) patients. There was male preponderance in both positive cases like 1.55:1 and 1.49 : 1, respectively. (Figure 1) The study analysis showed that 17.43% blood culture were positive, 78.79% were sterile and in 2.45% there was contamination which was less than 3% as cut off value as per national reporting.

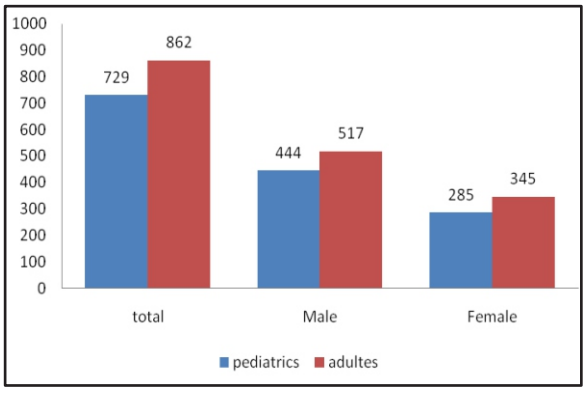


Figure 1: Blood Cultures received (1591) in the laboratory in paediatric and adults Patients

As per Table 3, around 18.30% (293/1598) bacteremias were due to gram positive cocci, gram negative bacilli and fungemia due to Candida spp. Maximum infections (66.21%) were due to gram negative bacilli, followed by gram positive cocci (32.42%) and then due to Candida species(1.36%).

Table 3: Isolates causing Blood Stream Infection (n=1598)

Sr. No.	Type of organism grown	Number	%
1	GPC	95	32.4 %
2	GNB	194	66.21%
3	Fungus (Candida)	04	1.36%
	Total	293	18.30%

Amongst the gram negative bacilli, non-lactose fermenting (NLF) bacilli were 57.21% (111/194) and the lactose fermenting bacilli were 42.78%. Out of the 57.21% of the NLFs isolated, 26.28% were Burkholderia *cepecea*, 17.01% were Pseudomonas *sp* and 6.18% were Sphingomonas *paucimobilis*. Other NLF isolates were Acinobacter (5.6%), Proteus *mirabilis*(0.51%) and Salmonella (1.03%). Lactose fermenting (LF) Gram negative bacilli isolated were Enterobacter *cloacae*(1.54%), E. *Coli* (23.91%) and Klebsiella *pneumonae*(18.04%). (Table-4)

Table 4 : Isolated Gram-Negative Bacilli from Blood Culture (n=194)

NLF Organism	Number of isolates	(%)
Acinetobacter sp.	11	5.6
Burkholderia cepacia	51	26.28
Proteus mirabilis	1	0.51
Pseudomonas sp.	33	17.01
Salmonella Sp.	2	1.03
Sphingomonas paucimobilis	12	6.18
Leclercia adecarboxylata	1	0.51
Total	**111**	**57.21**
LF Organism	**Number of isolates**	**(%)**
Enterobacter cloacae	03	1.54
E Coli	45	23.19
Klebsiealla Pneumonie	35	18.04
Total	**83**	**42.78**

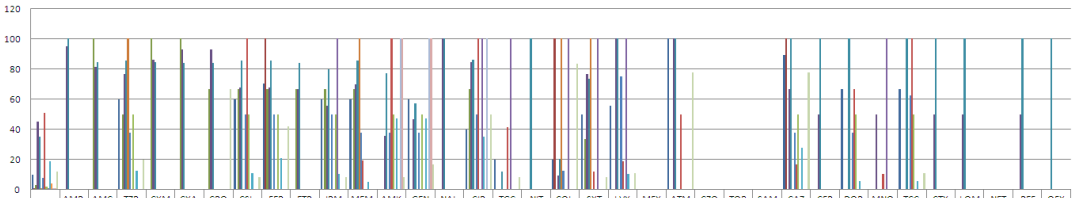
Out of the 95 isolates of gram positive cocci, we found that 25.26% Staphylococcus *hominis ss. hominis*, 21.05% were Staphylococcus *haemolyticus*, 16.84% were Staphylococcus *aureus ss. aureus*. Total methiciline resistance amongst Staphylococci was 85.33%. It was surprisingly noted that other species of Staphylococci also showed methyciline resistance. (Table 5)

Table 5: Gram positive cocci (n=95)

Organism	Number of isolates	(%)	MRSA	
			TOTAL	%
Enterococcus faecalis	1	01.05	-	-
Enterococcus faecium	4	04.21	-	-
Enterococcus gallinarum	1	01.05	-	-
Enterococcus sp.	10	10.50	-	-
Staphylococcus aureus ss. aureus	16	16.84	08	12.5
Staphylococcus capitis ss. capitis	1	01.05	01	1.5
Staphylococcus epidermidis	14	14.73	12	18.7
Staphylococcus haemolyticus	20	21.05	19	29.6
Staphylococcus hominis ss. hominis	24	25.26	21	32.8
Staphylococcus xylosus	1	01.05	01	1.5
Staphylococcus, coagulase negative (CONS)	3	3.157	02	3.1
TOTAL	**95**		**64**	**85.33**

Antibiotic resistance was reported based on the minimum inhibitory concentration (MIC) of the drugs. The Acinetobacters isolated belonged to baumanni sp. and general species. A. *baumanni* showed 100% resistance to aztreonam, 88% resistance to ceftazidime. There was a range of resistance from 50 to 70% to levofloxacin, sulfamethoxazole/trimethoprim, piperacillin/tazobactam, ticarcillin/clavulanic acid, and carbapenems. There was one Acinetobacter *sp*. which was 100% resistance to colistin, cefipine and ceftazidime. E *coli*, Klebsiealla *pneumonia*, Enterobacter *cloacae* showed 50 to 100% resistance to most of the antibiotics. *Leclercia adecarboxylata* is an aerobic gram-negative rod-shaped bacterium of the Enterobacteriaceae family and causes bacteremia's and respiratory tract infections. This is an unusual finding in our study and its antibiotics sensitivity testing showed that it is 100% resistance to piperacillin/tazobactam, meropenem, colistin and trimethoprim /sulfamethoxazole. The Pseudomonas sp. were sensitive to most of the antibiotics in the panel. Pseudomonas *aeruginosa*, Pseudomonas *fluorescens*, Pseudomonas *stutzeri* showed around 50% resistance. Burkholderia *cepacia* was 100% resistance to cefoperzone/sulbactam, amikacin, ciprofloxacin, ticarcillin/clavulanic acid and carbapenems. But it differed in showing only 12-19% resistance to meropenem, levofloxacin, and ceftazidime. Surprisingly, Proteus *mirabilis* was 100% resistance to imipenem, ciprofloxacin tigecycline, colistin, levofloxacin and minocycline. Isolated Salmonella *typhi* and Salmonella *para typhi* A were 100% resistance to amikacin, gentamicin and ciprofloxacin. (Table-6)

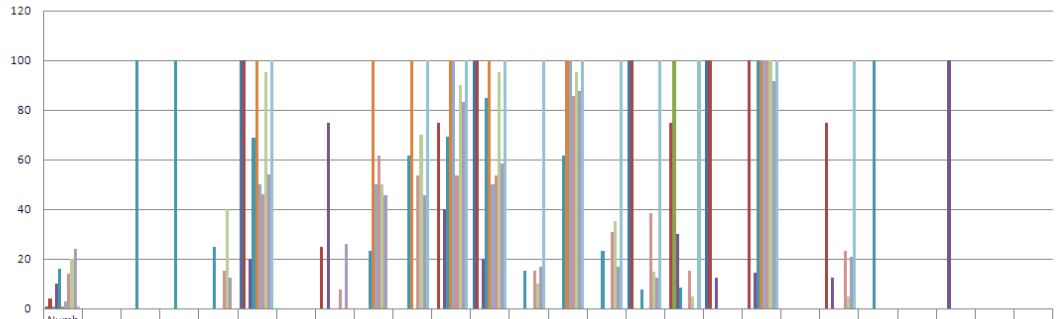
Table - 6 : NLF-LF Antibiotic Resistance



No:	AMP %R	AMC %R	TZP %R	CXM %R	CXA %R	CRO %R	CSL %R	FEP %R	ETP %R	IPM %R	MEM %R	AMK %R	GEN %R	NAL %R	CIP %R	TGC %R	NIT %R	COL %R	SXT %R	LVX %R	MFX %R	ATM %R	CZO %R	TOB %R	SAM %R	CAZ %R	CFP %R	DOR %R	MNO %R	TCC %R	CTX %R	LOM %R	NET %R	PEF %R	OFX %R
Acinetobacter baumannii	10			60			0	60	70		60	60		60		40	20		20	50	55.6		100				88.9		66.7	0	66.7					
Acinetobacter sp.	1			0				0	100		0	0	0	0		0	0		100	0	0						100		0	0	0					
Enterobacter cloacae	3		100	50	100	100	66.7	66.7	66.7	66.7	66.7	66.7	0	0	0	66.7	0	0	0	33.3																
Escherichia coli	45	94.9	81	76.7	86	92.5	92.5	67.4	67.4	66.7	55.8	69.8	35.6	46.7	100	84.4	0	0	9.3	76.7	100		100				66.7	50	0	50	0	50	50	0	50	0
Klebsiella pneumoniae ss. pneumoniae	35	100	84.4	85.3	84.4	83.9	83.9	85.3	85.3	83.9	79.4	85.3	77.1	57.1	100	85.7	11.8	100	20.6	73.5	100		100				100	100	100	0	100	100	100	0	100	100
Leclercia adecarboxylata	1			100				0	0		0	100	0	0		0	0		100	100	0														
Pseudomonas aeruginosa	8			37.5				50	50		50	37.5	37.5	37.5		50			12.5		75						37.5		37.5		62.5				
Burkholderia cepacia	51							100			0	19.6	100			100	41.2		0	12	19.1		50				17		66.7	10.6	100				
Pseudomonas fluorescens	2			50				50	50		50	0	50	50		0	0			0	0						50		50	0	50				
Proteus mirabilis	1			0				0	0		100	0	0	0		100	100		100	100	100		0				0			100	0				
Pseudomonas sp.	19			12.5				11.1	21.1		10.5	5.3	47.4	47.4		35.3	0		0	0	10.5				0		27.8		5.9	0	5.6				
Pseudomonas stutzeri	4			0				0	0		0	0	0	0		0	0			0	0						0		0	0	0				
Salmonella Paratyphi A	1			0				0	0		0	0	100	100		100	0		0	0	0		0				0		0	0	0				
Salmonella Typhi	1			0				0	0		0	0	100	100		0	0		0		0	0	0	0	0		0		0	0	0				
Sphingomonas paucimobilis	12			20			66.7	8.3	41.7		8.3	0	8.3	16.7		50	8.3		83.3	8.3	11.1		77.8				77.8		0	0	11.1				

AMP Ampicillin	CXM-Cefuroxime	AMK-Amikacin	GEN-Gentamicin	CIP-Ciprofloxacin	TGC-Tigecycline	OXA-Oxacillin	RIF - Rifampin	TCY-Tetracycline	VAN-Vancomycin	GEN-Gentamicin	PEN-Penicillin G
NIT-Netilmicin	SXT-Trimethoprim/Sulfamethoxazole	CLI-Clindamycin	ERY-Erythromycin	LVX - Levofloxacin	LNZ-Linezolid	DAP-Daptomycin	TEC - Teicoplanin	AZM-Azithromycin	NOR-Norfloxacin	FOS-Fosfomycin	SPX-Sparfloxacin

Table - 7 :GPC Antibiotics Resistance



	Number of isolates	AMP %R	CXM %R	AMK %R	GEN %R	CIP %R	TGC %R	NIT %R	SXT %R	CLI %R	ERY %R	LVX %R	LNZ %R	OXA %R	RIF %R	TCY %R	VAN %R	GEH %R	PEN %R	DAP %R	TEC %R	AZM %R	PRI %R	NOR %R	FOS %R	SPX %R
Enterococcus faecalis	1					100	0	0			0	100	0			100	0	100	0	0	0					
Enterococcus faecium	4					100	0	25			75	100	0			100	75	100	100		75					
Enterococcus gallinarum	1					0	0	0			0	0	0			0	100	0	0		0					
Enterococcus sp.	10	0			0	20	0	75			40	20	0			0	30	12.5	14.3		12.5		0	100	0	
Staphylococcus aureus ss. aureus	16	0	100	100	25	68.8	0	0	23.1	61.5	69.2	84.6	15.4	61.5	23.1	7.7	8.3		100	0	0	100				0
Staphylococcus capitis ss. capitis	1				0	100	0	0	100	100	100	100	0	100	0	0	0		100		0					
Staphylococcus, coagulase negative	3				0	50	0	0	50	0	100	50	0	100	0	0	0		100	0	0					
Staphylococcus epidermidis	14				15.4	46.2	0	7.7	61.5	53.8	53.8	53.8	15.4	85.7	30.8	38.5	15.4		100	0	23.1					
Staphylococcus haemolyticus	20				40	95	0	0	50	70	90	95	10	95	35	15	5		100	0	5					
Staphylococcus hominis ss. hominis	24				12.5	54.2	0	26.1	45.8	45.8	83.3	58.3	16.7	87.5	16.7	12.5	0		91.7	0	20.8					
Staphylococcus xylosus	1				0	100	0	0	0	100	100	100	100	100	100	100	100		100		100					

AMP-Ampicillin	AMC-Amoxicillin/ Clavulanic acid	TZP-Piperacillin/ Tazobactam	CXM-Cefuroxime	CXA-Cefuroxime axetil	CRO-Ceftriaxone	CSL-Cefoperazone/ Sulbactam	FEP-Cefepime	ETP-Ertapenem	IPM-Imipenem	MEM-Meropenem	AMK-Amikacin
GEN-Gentamicin	NAL-Nalidixic acid	CIP-Ciprofloxacin	TGC-Tigecycline	NIT-Netilmicin	COL-Colistin	SXT-Trimethoprim/ Sulfamethoxazole	LVX-Levofloxacin	MFX -Moxifloxacin	ATM-Aztreonam	TOB-Tobramycin	SAM -Ampicillin -Sulbactam
CAZ-Ceftazidime	CFP-Cefoperazone	DOR-Doripenem	MNO-Minocycline	TCC-Ticarcillin/ Clavulanic acid	CTX-Cefotaxime	LOM-Lomefloxacin	NET-Netilmicin	PEF-Pefloxacin	OFX-Ofloxacin		

Resistogram of Gram-Positive Cocci to different antibiotics is given in Table 7. Enterococci species were resistant to ciprofloxacin, nitrofurantoin, erythromycin, levofloxacin, gentamycin, penicillin and teicoplanin ranging from 0-75%. Enterococcus *faecium* (75%) and Enterococcus *gallinari* (100%) were resistant to vancomycin (VRE). Different species of staphylococcus identified were Staphylococcus *capitis*, CONs, Staph.*epidermidis*, Staph.*haemolytics*, Staph.*hominis* and Staph.*xylosus*. Staphylococcus *capitis* and *xyloses* were resistant to almost all antibiotics like ciprofloxacin, trimethoprim/ sulfamethoxazole, clindamycin, erythromycin, levofloxacin, oxacillin (MRSC,MRSX) and penicillin, but Staphylococcus *xyloses* was also resistant to tigecycline and vancomycin.

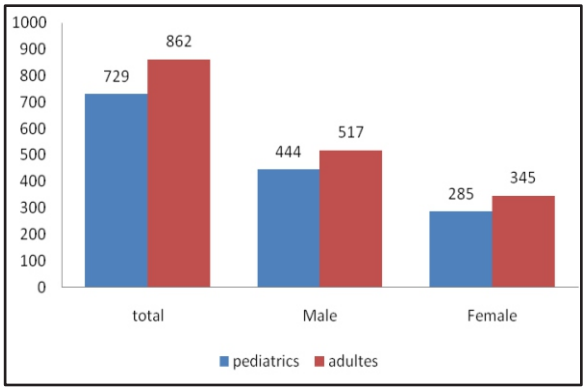


Figure 2: Sensitivity pattern of Candida sp.(n=3)

Candidemia was there in four patients and in three patients it was due to Candida *albicans* and in one patient it was due to C.*glabrata*. Both the species of Candida were sensitive to amphotericin, capsofungin, flucytocine, microfungin. C.*grabrata* was sensitive to Voriconazole and while C.*albicans* was resistant. (Figure 2)

Discussion

Approximately 85% of patients diagnosed as acute leukemia undergoing intensive anti-leukemia treatment developed infections and fever during neutropenic phase; and in 50% of these patients clinico-mcrobiological evidence of infection can be obtained specifically in cases of bacterimias.[3] This is basically due to the long-term placement of I.V. central line /peripheral catheters. The automated blood culture system from Becton Dickinson is a boon to the fast diagnosis of bacterimias and hastens up antibiotic treatment.

In the present retrospective analysis, it was observed that in 30.66 events the blood cultures were positive in one day (18-24 hours) for the detection of bacterial isolates which is much similar (19.33 hours) to the study conducted by Kaur et al (2004) from MM Institute of Medical Sciences & Research, Ambala.

Blood cultures are the strong supporting modality to guide the clinicians for better and timely antibiotic treatment in cases of febrile neutropenia.

There were 64.5 % requests from clinicians for leukemia patients with symptoms of septicemias & there has always been male preponderance in pediatric as well as adult patients. Out of which 66.2% of bacteremias were due to gram negative bacilli (GNB) and 32.4% due to gram positive cocci (GPC) unlike the study of Kaur et al (2014), who reported 19.06% GNB & 19.33 GPC isolated.

In a review of epidemiology and antibiotic resistance the recent changes in bacteremia in patients with cancer found that gram negative bacteria were the most frequent pathogens isolated. They also found the extensive emergence of antimicrobial resistant strains associated with increased risk of morbidity. This increasing incidence of antibiotic resistance was reported in both types of organism.[2] A similar picture was highlighted in our data analysis too, where in non–lactose fermenting bacilli showed 50-100% resistance to cephalosporins, aminoglycosides and beta lactam and beta lactamase inhibitors.

Some of the un-usually isolated bacteria like *Leclercia adecarboxylata* were flagged in the blood cultures by the automated Vitek ID and AST system to identify the kind of organism. This bacterium was resistant to beta lactam and beta lactamase inhibitor combination unlike the reports published in the literature. Unlike the study by Kaur et al[3] where they found 29.92% CONS, our observation showed Staphylococcus *Hominis sp Hominis* were highlighted more. Staphylococcus *hominis* is a human skin contaminant. It causes infections in people with abnormally weak immune system and majority of our patients are immunocompromised.

The methicillin resistant Staphylococcus *species*(MRSsp) identified were 85.53% of which Staphylococcus *hominis* showed 32.8%, Staphylococcus *haemolyticus* showed 29.6% and Staphylococcus *epidermis* showed 18.7% resistance to methicillin. Staphylococcus *aureus* showed 12.5% resistance to methicillin(MRSA). Our study differed a lot from the one conducted by Olson et al[5] where they communicated that methicillin resistant S. epidermidis(MRSE) accounted for 47% and methicillin resistant S. aureus was 29.5%. Majority of the isolation was in patients with ophthalmic disease. Their study was limited to two species of Staphylococci, epidermidis & aureus. In recent study by Asai *et al* (2020)[6] where they characterized relevance of coagulase-negative Staphylococcus epidermidis by automated blood culture system from BD, and identified using MALDI-TOF instrument. They found that most common isolates were Staphylococcus *hominis* (38%), followed by S. *capitus* (24%) and S. *caprae* (16%). Their study is approximately comparable to current study where S. *hominis* isolation was almost similar.

Conclusion

In conclusion it was found that automated blood culture system is convenient, simple to use and rapid method for the diagnosis of septicemias, bacteremia and fungemia. In this study analysis, it is observed that gram negative bacteria especially the *Enterobacteriaceae* are the major cause of bacteremia in hematology and other oncology patients in our institute. The high antibiotic resistance among the gram negative and gram-positive microorganisms is seen, for which combination therapy of aminoglycosides with cephalosporins and piperacillin/tazobactam can be given. For the gram-positive cocci antibiotic vancomycin must be spared to treat critically ill patients and linezolid is to be given for resistant gram-positive infection. The emerging trends in antibiotic resistance, and the spread of methicillin resistance to oppurtunistic staphylococci infections is a major concern and calls for vigilence in choosing antibiotics for gram positive cocci to treat patients and further the multi drug resistance of gram negative bacilli is again a concern and requires to formulate empirical therapy and warns us to create antibiotic policies for the hospital and should be made available in consultation with the clinicians where patients are getting infected with superbugs.

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