Ecology of Bloodstream Infections and Temporal Trends of Their Antibiograms with Respect to Source and Duration of Incubation: A 5-Year Retrospective Observational Analysis

Amit Banik[1], Valarie W. Lyngdoh[2] Elantamilan Durairaj[2] Anil C. Phukan[2] Raghavendra Kotal[3]

[1]All India Institute of Hygiene & Public Health, Kolkata, West Bengal, India
[2]Department of Microbiology, NEIGRIHMS, Shillong, Meghalaya
[3]Department of Anaesthesiology & Critical Care, NEIGRIHMS, Shillong, Meghalaya

Address for correspondence Amit Banik, MBBS, MD, DNB, All India Institute of Hygiene & Public Health, Room# 203, AIIH&PH, BN Campus, Kolkata, West Bengal 700106, India (e-mail: dramitbanik@gmail.com).

J Lab Physicians:2020;12:56–67

Abstract

Purpose Blood is one of the most important connective tissues of human body. Bloodstream infection can range from inapparent bacteremia till fulminant septic shock with high mortality. Presence of microbes in blood whether continuously, intermittently, or transiently is a grave risk to every organ of body. Culture of blood is a vital tool to diagnose such infections. Drug susceptibility patterns help in rationalizing therapy.

Objective The aim of the study is to perform bacteriological analysis and assess drug sensitivity patterns of blood culture isolates and compare in light of other associated variables.

Design Retrospective observational study was conducted from January 2009 to December 2013 at a tertiary care hospital at Shillong, India. Blood samples were collected with aseptic guidelines and cultured for 7 days. Growths were identified by standard biochemical tests and subjected to sensitivity testing according to Modified Kirby Bauer disk diffusion method. Data for source of blood collection and duration of incubation were noted and compared.

Results A total of 658 (11.2%) pathogens were isolated from 5,867 bacteremia-suspected patient blood specimens. Contamination was observed at the rate of 1.21%. Gram-negative organisms were the predominant pathogens recovered, *Klebsiella pneumoniae* being the most common. No significant difference was observed between the number of organisms isolated within or beyond 48 hours. *Acinetobacter baumannii* and *K. pneumoniae* have significantly higher chances ($p < 0.05$) of isolation from central line catheters compared with peripheral venipuncture.

Conclusion Successful treatment of sepsis depends on early diagnosis and proper antimicrobial therapy. Local knowledge of bacteriological profile and antimicrobial sensitivity patterns helps rationalize empiric treatment strategies.

Keywords
- bloodstream infection
- Meghalaya
- antimicrobial sensitivity
- blood culture
- empirical therapy

Introduction

Blood is in the truest sense the elixir of life. It contains a part of the extracellular fluid along with a wide variety of other constituents which are indispensable for proper functioning and survival of human life. From providing nutrients, limiting pathogens, perfusing and ventilating organs, clotting wounds, removal of toxins and chemicals, and dissemination of hormones and drugs throughout the body it performs a pivotal role in body defense and survival. Presence of organisms in blood can give rise to different clinical scenarios. Clinical presentation ranges from benign transient bacteremia with

DOI https://doi.org/10.1055/s-0040-1714199
ISSN 0974-2727 .

©2020 by The Indian Association of Laboratory Physicians

License terms

little or no symptoms to fulminant septic shock with high mortality.[1]

Bacteremia or fungemia denotes the presence of viable bacteria or fungi in the blood with or without clinical symptoms. Systemic inflammatory response syndrome (SIRS) is defined by the presence of two or more acute findings (tachycardia, leukocytosis, or leukocytopenia, fever or hypothermia, tachypnea). Combination of SIRS and bacteremia is known as sepsis which is a host systemic response to infection. Sepsis with additional acute organ dysfunction due to documented or suspected infection is known as severe sepsis. Septic shock is defined as severe sepsis with hypotension which is unresponsive to fluid resuscitation.[2] Blood stream infections (BSI) are a major cause of morbidity and mortality and are among the most common health care-associated infections. With an attributable mortality rate of 15%, it is an eminent cause of death worldwide.[1]

Presence of microbes in blood whether continuously, intermittently, or transiently is a grave risk to every organ of the body. Early diagnosis plays a crucial role in managing BSI and hence prompt detection of such infections is a critical function of clinical microbiology laboratories. Blood culture is a vital tool for the detection of BSI and is the gold standard for bacteremia detection. Initial antimicrobial empirical therapy being very imperative in BSI, must be based on the knowledge of the bacterial profile and their sensitivity patterns.[3] Irrational use of drugs leads to an increase of multidrug-resistant bugs and thus worsens the management of the infections. Prevalence and susceptibility patterns of microorganisms vary according to the geography and use of antibiotics in different health care settings. There is paucity of similar reports with regard to disease burden from Northeast India, and more so from Meghalaya. This region is unique with respect to its ethnicity, geographical location, topography, climatic condition unlike the rest of the country. The current study intends to report the prevalence, bacteriological analysis of microorganisms, and antimicrobial susceptibility profiles of blood culture isolates and other auxiliary variables in a tertiary health care center in Northeast India.

Materials and Methods

The present study is a 5-year retrospective observational analysis of blood culture isolates received in the Department of Microbiology of the hospital from January 2009 to December 2013. Necessary Ethics Committee Approval was obtained for the study.

Sample Collection

Blood specimens were obtained at bedside either by a trained phlebotomist or by nursing staff from wards and critical care units. The skin was disinfected with 2% chlorhexidine. Approximately 5 to 10 mL of blood was collected from adult patients, 1 to 5 mL from pediatric patients, and 1 to 2 mL from neonates. The antecubital and median cubital fossa were the preferred sampling sites using a needle and syringe. The blood samples from the central vein catheters were obtained from needleless caps that have been disinfected with 70% isopropyl alcohol, allowed to dry, and wiped with sterile gauze prior to obtaining the sample.

Sample Processing

Blood for culture was collected from 5,867 clinically suspected bacteremia cases under strict aseptic precautions. They were inoculated into conventional blood culture bottles containing Brain Heart Infusion broths (1:10 dilution). These were incubated aerobically at 37°C, observed for turbidity every morning, and manually agitated for aeration for 7 complete days.[4] Regular blind subcultures were done at the completion of day 2 and day 7 of aerobic incubation. Subcultures were done on MacConkey agar, 5% Sheep blood agar, and chocolate agar as and when turbidity was noticed. Specimens were discarded after 7 days of unsuccessful aerobic incubation.[5] Any growth obtained was processed and identified by Gram staining, colony morphology, and standard biochemical tests. Antibiotic susceptibility testing was done according to Kirby Bauer disk diffusion method and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[6] Positive growths were further critically analyzed based on the criteria to be agents of bacteremia, fungemia, and contaminants.[7-9] The data were manually compiled and analyzed critically for the study. The following strains were used as quality control strains:

1. *Staphylococcus aureus* (ATCC 25923)
2. *Escherichia coli* (ATCC 25922)
3. *Pseudomonas aeruginosa* (ATCC 27853)

Results

The present study involves 5,867 continuous samples received from different wards and intensive care units (ICUs) of the hospital. Among them, 740 isolates showed positive aerobic bacterial growth and 669 (11.40%) of them were recognized pathogens. However, blood bank surveillance revealed 11 pathogens and 17 contaminants. Finally, 658 (11.2%) isolates were recovered from patients as incriminating microorganisms responsible for bacteremia/fungemia. More males suffered from bacteremia than females with a gender ratio skewed at 1.4:1, (►**Supplementary Table S1**, online only). The mean age of patients was 33.67 ± 23 years (range 0–85 years). Bulk of the specimens were sent from Medicine, Pediatric wards apart from critical care units. Data including the most common clinical syndromes leading to bacteremia/fungemia and the corresponding distribution of the microbial agents responsible are depicted in ►**Table 1**. Cerebrovascular accidents and their subsequent complications were the leading cause for BSIs. Contamination in blood culture was documented in 71 (1.21%) isolates in the present study. Most of them were from blood bank and pediatric ICU. A detailed analysis of contaminants obtained from different sections of hospital is presented in ►**Table 2**. Among 658 isolates recovered from patients, the spectrum of microbes included 436 (66.3%) gram-negative bacilli (GNB), 195 (29.6%) gram-positive cocci (GPC), 15 gram-negative cocci, 1 gram-positive bacilli,

Table 1 Most common clinical syndromes with incriminating microbes' distribution

Sl. no.	Name of initial clinical diagnosis/ syndrome	Microbes isolated No. of cases (n =?)	*Staphylococcus spp.*	*Enterococcus spp.*	*Klebsiella spp.*	*Escherichia coli*	*Pseudomonas spp.*	*Acinetobacter spp.*	*Enterobacter spp.*	*Nonfermenting* GNB	*Neisseria meningitidis*	*Salmonella spp.*	Others
1	Severe pneumonia	12	4			2	2	2					2
2	DM with complications	21	2	1	6	7		1	2	1			1
3	Tuberculous meningitis	15	3		5		1	4		2			
4	Bacteremia and sepsis	14	5	1		3	2	2		1			
5	Seizures and epilepsy	08	3					3	1				1
6	Rheumatic disease with complications	14		5	3	1	1	2		1			1
7	Prematurity	9	2		2		3	1	1				
8	Postoperative infection (general surgical)	27	4		3	6	8	3		2			1
9	Post exploratory laparotomy	23	2	1	4			9		6			1
10	Meningitis and meningoencephalitis	28	4	1	2		1	1	1	3	13		2
11	Intracranial hemorrhage	16	1		11		1		3				
12	Hepatic encephalopathy	8			1	1	3		1				2
13	CKD with complications	26	1		8	4	3	10					
14	Acute pancreatitis	10	2		5		2						1
15	CVA with complications	49	3	1	18		5	15	3	1			3
16	Enteric fever	6		1				1				4	
17	COPD with complications	8	2	1				4					1
18	Fever	11	3			1	2			1		4	

(*Continued*)

Table 1 (*Continued*)

Sl. no.	Name of initial clinical diagnosis/ syndrome	Microbes isolated		*Staphylococcus spp.*	*Enterococcus spp.*	*Klebsiella spp.*	*Escherichia coli*	*Pseudomonas spp.*	*Acinetobacter spp.*	*Enterobacter spp.*	*Nonfermenting* GNB	*Neisseria meningitidis*	*Salmonella spp.*	Others
			No. of cases (*n* =?)											
19	Fever under evaluation	38		13	1			4	6	2	3		1	8
20	Uncertain diagnosis	22		5	2	7	1	1	3			1	1	1
21	CHD with CCF	8		4		1			1					2
22	CIDP with autonomic neuropathy	8				3	1	1	3					
23	Hypertensive disorders with complications	13		1	2			1	8					1

Abbreviations: CCF, chronic congestive failure; CHD, chronic heart disease; CIDP, chronic inflammatory demyelinating polyneuropathy; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary diseases; CVA, cerebrovascular accident; DM, diabetes mellitus; GNB, gram-negative Bacilli.

and 11 yeasts. Isolation of GNBs was significantly higher ($p < 0.001$) than other groups of organisms. Within GNBs, *Klebsiella pneumoniae* (28.67%) was the dominant isolate obtained followed by *Acinetobacter baumannii* (22.47%) and *Pseudomonas aeruginosa* (14.45%). Eleven isolates of *Salmonella spp*. and one isolate of *Hemophilus influenzae* were also recovered. As a group, *Enterobacteriaceae* comprised half of all GNBs and 32.67% of total pathogens recovered. Among GPCs isolated, *S. aureus* (65.6%) was the dominant organism followed by *Enterococcus spp.* (15.38%) and *Streptococcus* spp. (11.8%). One-third of *Enterococcus spp.* isolates carried high-level aminoglycoside resistance (HLAR) genes. *Streptococcus pneumoniae* was recovered from seven bacteremic patients. Of them, five were children younger than 9 years and two elderly older than 50 years. They were most likely severe cases of pneumococcal pneumonia causing bacteremia. Fifteen isolates of *Neisseria meningitidis* were recovered from the study. *Neisseria meningitidis* is a dreaded organism considering the capability of this organism to rapidly deteriorate relatively benign bacteremia into fulminant bacteremia and septic shock. Worth mentioning here is that all these cases were recovered during an outbreak between November 2008 and April 2009.

Fungemia confirmed in 11 cases were mostly *Candida spp.* A single case of bacteremia by *Listeria monocytogenes* was also detected in a 2-year-old child who presented with acute gastroenteritis. Comparison of isolation rates of organisms on different days of incubation shows that chances of isolation of microbes within the second day of incubation was significantly ($p < 0.001$) higher than other days. The distribution of different isolates and groups recovered with each passing day of incubation is presented in ►**Table 3**. Significant isolation ($n = 242$, 36.7%) of organisms was obtained subsequent to turbidity detection on the second day. This was followed by higher detection of organisms on the third day ($n = 115$) and first day ($n = 89$) of incubation. A total of 331 isolates were recovered after detecting turbidity within the first 48 hours of aerobic incubation compared with 327 isolates of organisms which were recovered when incubation was continued beyond 48 hours till 7 days. Interestingly, both *K. pneumoniae* and *Escherichia coli* were consistently isolated better in significant proportions ($p < 0.001$) within the first 48 hours. Almost 73.8 and 80% isolates of *K. pneumoniae* and *E. coli*, respectively were detected within initial 48 hours. Concurrently, organisms which have significantly higher chances of recovery after initial 48 hours include *Candida spp.* ($p = 0.006$), *N. meningitidis* ($p = 0.017$), *Salmonella paratyphi* ($p = 0.044$), methicillin-resistant *S. aureus* (MRSA; $p < 0.001$), and methicillin-sensitive *S. aureus* (MSSA, $p = 0.024$). However, there was no significant difference between number of organisms isolated within or beyond 48 hours till 7 days. A comprehensive distribution frequency table shows different microbes with their growth patterns at 48 hours and at 7 days (►**Table 4**). A comparison of data about the source of blood collection shows that *A. baumannii* and *K. pneumoniae* have significantly higher chances ($p < 0.05$) of isolation from central line catheters compared with peripheral venipuncture, while

Table 2 Details of samples, pathogens, and contaminants from different wards

Department	Total samples	Negative culture samples	Organisms isolated		Pathogen yield		Contamination	
				(%)		(%)		(%)
Blood bank	181	153	28	15.47	11	6.08	17	9.39
Cardiology	326	315	11	3.37	10	3.07	1	0.31
Medicine	1,236	1,162	74	5.99	64	5.18	10	0.81
CTVS	147	127	20	13.61	19	12.93	1	0.68
Dialysis unit	8	6	2	25.00	2	25.00	0	0.00
Otolaryngology	16	16	0	0.00	0	0.00	0	0.00
General surgery	40	38	2	5.00	1	2.50	1	2.50
Coronary care unit	172	155	17	9.88	17	9.88	0	0.00
ICU	1,170	800	370	31.62	362	30.94	8	0.68
Neurology	60	59	1	1.67	1	1.67	0	0.00
Obstetrics and gynecology	143	134	9	6.29	8	5.59	1	0.70
Oncology	42	42	0	0.00	0	0.00	0	0.00
Ophthalmology	2	1	1	50.00	0	0.00	1	50.0
Orthopedics	45	39	6	13.33	5	11.11	1	2.22
Pediatrics	911	836	75	8.23	65	7.14	10	1.10
Pediatric ICU	1,239	1,122	117	9.44	98	7.91	19	1.53
Private ward	84	79	5	5.95	4	4.76	1	1.19
Urology	45	43	2	4.44	2	4.44	0	0.00
Total	**5,867**	**5,127**	**740**	**12.61**	**669**	**11.40**	**71**	**1.21**

Abbreviations: CTVS, cardiothoracic and vascular surgery; ICU, intensive care unit.

chances of recovery of isolates from peripheral venipuncture were observed to be significantly higher for *Candida spp.*, *Enterococcus* (HLAR) spp., *Salmonella typhi, S. aureus, S. pneumoniae*, and other α and β hemolytic *Streptococcus spp*. A description about the same attribute is depicted in ►**Table 5**. The antibiotic susceptibility patterns for GPCs and GNBs were interpreted in accordance to prevalent CLSI guidelines[6] and are represented in ►**Tables 6** and **7**, respectively.

Beta lactams proved least effective for GPCs with sensitivity lowest in penicillin (29.6%), ceftriaxone (45.6%), and cephalexin (52.6%). Erythromycin (31.7%) and rifampicin (48.6%) were largely ineffective. Relative sensitivity was highest for vancomycin (96.5%) and linezolid (90.2%). Even broad-spectrum antibiotics like chloramphenicol (78.5%) and tetracycline (74.8%) had good sensitivity against GPCs, especially MRSA. MSSA isolates uniformly showed very high sensitivities of > 85% to ampicillin, gentamicin, amikacin, tetracycline, chloramphenicol, levofloxacin, and cefotaxime. *Streptococcus pneumoniae* showed an incredibly good sensitivity to amoxicillin (100%), cefepime (100%), and penicillin (80%).

Neisseria meningitidis isolates showed high sensitivity to penicillin (81.8%), azithromycin (75%), and ceftriaxone (66.67%). Interestingly, ciprofloxacin (41.67%) was only moderately effective. This is important because ciprofloxacin was the antimicrobial initially used to control the outbreak and also for the prophylaxis of contacts.

For GNBs, carbapenems were the most effective drugs (approximately > 85% sensitivity). Cefoperazone-sulbactam (CFS, 83.1%) was the only other drug with sensitivity ≥80%. Quinolones were much less effective. Injectables like gentamicin (58%) and amikacin (63%) showed moderate sensitivity. Cephalosporins were mostly ineffective with sensitivities as low as 10%. *K. pneumoniae* and *E. coli* were highly susceptible to carbapenems (≥95% sensitivity) and CFS. *E. coli* additionally had better susceptibility (≥85%) against aminoglycosides too. Quinolones were moderately effective for *Klebsiella spp.* (60–75% sensitivity) but serve as very poor drugs to treat *E. coli* (15–20% sensitivity) BSIs. *A. baumannii* was most sensitive to carbapenems, CFS, moderately sensitive to quinolones, gentamicin, piperacillin-tazobactam. However, *Acinetobacter lwoffii* documented better sensitivity to aminoglycosides and quinolones compared with carbapenems. *P. aeruginosa* isolated in 14.4% BSIs, was better managed with imipenem, CFS (> 80% sensitivity), piperacillin-tazobactam, and levofloxacin, whereas typical antipseudomonal drugs like cefoperazone and ceftazidime were largely ineffective. Nonfermenting GNBs (other than *Acinetobacter* and *Pseudomonas spp.*) were highly susceptible to quinolones, meropenem, CFS, piperacillin-tazobactam, and chloramphenicol. Imipenem and gentamicin were drugs which were moderately sensitive

Table 3 Day-wise distribution of blood stream pathogens recovered

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
Gram-positive cocci								
Staphylococcus aureus (MSSA)	5	20	13	12	4	7	6	67
Staphylococcus aureus (MRSA)	4	7	17	13	9	8	3	61
CoNS	0	3	2	1	0	1	0	7
Enterococcus spp.	3	9	4	0	0	3	1	20
Enterococcus spp. (HLAR)	2	4	0	1	1	0	2	10
α-hemolytic Streptococcus spp.	6	4	3	4	0	1	0	18
β-hemolytic Streptococcus spp.	0	2	2	0	0	0	1	5
Streptococcus pneumoniae	0	4	1	2	0	0	0	7
Total								***n* = 195**
Gram-negative bacilli								
Escherichia coli	11	17	4	1	1	1	0	35
Klebsiella oxytoca	2	2	1	0	0	0	0	5
Klebsiella pneumoniae	29	63	13	5	9	1	5	125
Nonfermenter GNB	3	11	1	6	6	4	4	35
Acinetobacter baumannii	14	39	12	12	9	2	10	98
Acinetobacter lwoffii	1	8	3	0	5	1	4	22
Proteus spp.	0	0	2	0	0	1	0	3
Proteus mirabilis	0	2	0	0	0	0	0	2
Morganella morganii	1	1	0	0	0	0	0	2
Enterobacter spp.	3	10	2	0	1	5	4	25
Citrobacter freundii	0	2	1	0	0	0	0	3
Citrobacter diversus	0	2	1	0	0	1	0	4
Pseudomonas spp.	0	0	0	0	1	0	1	2
Pseudomonas aeruginosa	3	26	16	10	1	4	3	63
Salmonella paratyphi	0	0	4	0	0	0	0	4
Salmonella enteritidis	1	0	0	0	0	0	0	1
Salmonella typhi	0	2	1	0	0	1	2	6
Hemophilus influenzae	0	1	0	0	0	0	0	1
Total								***n* = 436**
Others								
Neisseria meningitidis	1	2	8	0	1	1	2	15
Listeria monocytogenes	0	0	0	0	1	0	0	1
Candida spp.	0	1	4	0	4	2	0	11
GRAND TOTAL	89	242	115	67	53	44	48	**658**

Abbreviations: CoNS, coagulase negative *Staphylococcus spp*.; GNB, gram-negative bacilli; HLAR, high-level aminoglycoside resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.

to these organisms. *Morganella, Salmonella,* and *Hemophilus spp.* isolates recovered were completely susceptible to usual drugs tested and no major antimicrobial resistance trends were observed in them.

Discussion

BSI constitutes one of the major causes of morbidity and mortality. Definitive diagnosis is established by bacteriologic culture of blood samples to identify organisms and provide antimicrobial susceptibility.[10] Numerous analyses have concluded that early treatment of bacteremic patients with an appropriate antimicrobial drug improves survival.[11-14] There exists a strong relationship between delay in effective initiation of therapy and in-hospital mortality of septic shock. Each hour of delay in therapy initiation is associated with an average decrease in survival of 8%.[15] Defining pathogen distribution and drug resistance patterns provides basis for empirical as well as definitive therapy.[16] This present study is an attempt to analyze the bacterial

Table 4 Comparison of yield of pathogens after aerobic incubation between 48 h vs. 7 d

	Day				Total	Significance test	
	48 h		7 d				
	N	%	*N*	%		*Z*-Stat	*p*-Value
Acinetobacter baumannii	53	16.01%	45	13.76%	98	0.81	0.417
Acinetobacter lwoffii	9	2.72%	13	3.98%	22	−0.9	0.37
Candida spp.	1	0.30%	10	3.06%	11	−2.76	0.006
Citrobacter spp.	4	1.21%	3	0.92%	7	0.36	0.716
CoNS	3	0.91%	4	1.22%	7	−0.4	0.692
Enterobacter spp.	13	3.93%	12	3.67%	25	0.17	0.863
Enterococcus spp.	12	3.63%	8	2.45%	20	0.88	0.378
Enterococcus spp. (HLAR)	6	1.81%	4	1.22%	10	0.62	0.536
Escherichia coli	28	8.46%	7	2.14%	35	3.66	< 0.001
Hemophilus influenzae	1	0.30%	0	0.00%	1	−1	0.317
Klebsiella oxytoca	4	1.21%	1	0.31%	5	1.34	0.18
Klebsiella pneumoniae	92	27.79%	33	10.09%	125	5.95	< 0.001
Listeria monocytogenes	0	0.00%	1	0.31%	1	−1	0.317
Morganella morganii	2	0.60%	0	0.00%	2	1.42	0.156
Neisseria meningitidis	3	0.91%	12	3.67%	15	−2.38	0.017
Nonfermenter GNB	14	4.23%	21	6.42%	35	−1.25	0.21
Proteus spp.	2	0.60%	3	0.92%	5	−0.46	0.644
Pseudomonas aeruginosa	29	8.76%	34	10.40%	63	−0.71	0.476
Pseudomonas spp.	0	0.00%	2	0.61%	2	−1.42	0.156
Salmonella enteritidis	1	0.30%	0	0.00%	1	1	0.317
Salmonella paratyphi	0	0.00%	4	1.22%	4	−2.01	0.044
Salmonella Typhi	2	0.60%	4	1.22%	6	−0.83	0.404
Staphylococcus aureus (MRSA)	11	3.32%	50	15.29%	61	−5.39	< 0.001
Staphylococcus aureus (MSSA)	25	7.55%	42	12.84%	67	−2.25	0.024
Streptococcus pneumoniae	4	1.21%	3	0.92%	7	0.36	0.716
α-hemolytic *Streptococcus spp.*	10	3.02%	8	2.45%	18	0.45	0.651
β-hemolytic *Streptococcus spp.*	2	0.60%	3	0.92%	5	−0.46	0.644
Total	**331**	100.00%	**327**	100.00%	**658**		

Abbreviations: CoNS, coagulase negative *Staphylococcus spp.*; GNB, gram-negative Bacilli; HLAR, high-level aminoglycoside resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.

profile of blood culture isolates, assess antimicrobial trends, correlate bacteremic source and their impact, and discuss other variables which may help us devise the best ways of managing BSIs.

This is a 5-year retrospective analysis of 5,867 blood samples received from clinically suspected bacteremia patients. A total of 658 recognized pathogens were recovered from 637 specimens. A total of 97% isolates had monomicrobial growths. The rate of isolation for blood culture pathogens was observed at 11.2%. This is significant considering it is a new tertiary level 450-bedded hospital apart from the fact that the laboratory uses conventional blood culture bottles with no provision for continuous monitoring of culture bottles. Besides, this rate of isolation is in consonance with many studies from India[17-22] and abroad.[23-25]

Although the mean age of bacteremic patients in the present study was 33.7 years, the highest number of cases of sepsis and shock were observed in children younger than 5 years age group as well as adults from 21 to 40 years age bracket. After the age of 40 years, the incidence of bacteremia cases shows a steady decline with every passing decade. Neonates, infants, and young children are particularly vulnerable to BSI owing to numerous risk factors like premature rupture of membrane, prolonged rupture, prematurity, recurrent urinary tract infection, poor maternal nutrition, low birthweight, birth asphyxia, congenital anomalies, and nascent/weak host immunity.[10]

Almost 31% of all medical ICU patients suspected with bacteremia were confirmed by culture. This is understood as patients admitted in ICU are usually already severely ill who are put under continued and enhanced vigilant care. Since

Table 5 Comparative data about blood stream infections from central line vs. peripheral lines[a]

	Line				Total	Significance test	
	Central	%	Peripheral	%			
	n = 93		*n* = 395			*Z*-Stat	*p*-Value
Acinetobacter baumannii	26	27.96%	59	14.94%	85	2.6100	0.0090
Acinetobacter lwoffii	3	3.23%	12	3.04%	15	0.0900	0.9260
Candida spp.	0	0.00%	5	1.27%	5	−2.2500	0.0240
Citrobacter spp.	0	0.00%	2	0.51%	2	−1.4200	0.1560
CoNS	1	1.08%	3	0.76%	4	0.2700	0.7850
Enterobacter spp.	3	3.23%	16	4.05%	19	−0.4000	0.692
Enterococcus spp.	3	3.23%	12	3.04%	15	0.0900	0.9260
Enterococcus spp. (HLAR)	0	0.00%	6	1.52%	6	−2.4700	0.0140
Escherichia coli	3	3.23%	23	5.82%	26	−1.1900	0.2330
Hemophilus influenzae	0	0.00%	1	0.25%	1	−1.0000	0.3170
Klebsiella oxytoca	1	1.08%	2	0.51%	3	0.5000	0.4700
Klebsiella pneumoniae	31	33.33%	68	17.22%	99	3.0700	0.0020
Listeria monocytogenes	0	0.00%	1	0.25%	1	−1.0000	0.3170
Morganella morganii	1	1.08%	1	0.25%	2	0.7500	0.4540
Neisseria meningitidis	0	0.00%	1	0.25%	1	−1.0000	0.3170
Nonfermenting gram-negative Bacilli	4	4.30%	29	7.34%	33	−1.2300	0.2200
Proteus spp.	2	2.15%	3	0.76%	5	0.8900	0.3750
Pseudomonas aeruginosa	11	11.83%	39	9.87%	50	0.5300	0.5940
Pseudomonas spp.	0	0.00%	0	0.00%	0	NA	
Salmonella enteritidis	0	0.00%	1	0.25%	1	−1.0000	0.3170
Salmonella paratyphi	0	0.00%	3	0.76%	3	−1.7400	0.0820
Salmonella typhi	0	0.00%	4	1.01%	4	−2.0100	0.0440
Staphylococcus aureus (MRSA)	2	2.15%	38	9.62%	40	−3.5400	< 0.001
Staphylococcus aureus (MSSA)	2	2.15%	45	11.39%	47	−4.2100	< 0.001
Streptococcus pneumoniae	0	0.00%	5	1.27%	5	−2.2500	0.0240
α-Hemolytic *Streptococcus spp.*	0	0.00%	11	2.78%	11	−3.3600	0.0010
β-Hemolytic *Streptococcus spp.*	0	0.00%	5	1.27%	5	−2.2500	0.0240
	93	19.02%	395	80.78%	488		

Abbreviations: CoNS, coagulase negative *Staphylococcus* spp.; HLAR, high-level aminoglycoside resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.

[a]Data for 2011 to 2013 only.

they are better equipped to handle such serious infections, critical patients are referred to ICUs more frequently from other departments which explains the highest positivity yield of blood culture specimens received from medical ICU.

The rate of contamination observed (1.21%) is below the target level suggested by Hall and Lyman.[7] This correlates well with other studies by Archibald et al[26] and Weinstein.[9] These included mainly isolates of *Bacillus spp. Corynebacterium spp.*, and *Micrococcus spp*.

Among 658 pathogens isolated, GNB were significantly the predominant organisms. This corresponds to findings documented by other similar studies.[10,17,21] *Klebsiella spp.* as a dominant microbe causing BSIs was also reported by Roy et al[21] and Tariq.[23]

Within GPCs, *S. aureus* (65.6%) 'was the predominant pathogenic organism isolated.' Pre-eminence of *S. aureus* as a bloodstream pathogen has been documented by numerous similar studies.[10,18,25,27] If a comparison of methicillin sensitivity is attempted among *S. aureus* strains, 52.34% were MSSA and rest were MRSA. MRSA are notorious since these are resistant to action of a broad group of β-lactam antibiotics, which cannot be used for therapy. One-third (10/30) of *Enterococcus*

Table 6 Drug sensitivity profile of gram-positive strains

Drug	Overall GPC	*Enterococcus spp.*	*Enterococcus spp.* (HLAR)	MRSA	MSSA	*Streptococcus pneumoniae*	CoNS	α-Hemolytic *Streptococcus spp.*	*Neisseria meningitidis*
Imipenem	85.7	90.9	60.0						
Ciprofloxacin	57.3	58.3	0.0	39.6	76.3		50.0	100.0	41.7
Penicillin	29.6	35.7	100.0	10.3	100.0	80.0	33.3	77.8	81.8
Ampicillin	71.4	68.4	50.0		100.0			81.8	
Vancomycin	96.5	100.0	87.5	96.3		100.0	100.0	100.0	
Linezolid	90.2	90.0	100.0	96.3		100.0	66.7		
Gentamicin	79.8	86.7	0.0	65.3	97.9		80.0	80.0	
Tetracycline	74.8	42.9	20.0	83.3	87.2	50.0	25.0	100.0	
Amikacin	68.9	0.0	0.0	85.7	100.0				
Levofloxacin		33.3	0.0	60.0	91.3	50.0	100.0		
Cefotaxime	67.8	100.0	0.0	23.1	90.0	75.0	83.3	83.3	
Chloramphenicol	78.5		50.0	70.6	88.5	60.0	50.0	100.0	50.0
Ofloxacin	61.4			46.8	86.1		0.0		
Netilmicin	85.9			81.3	97.1				
Rifampicin	48.6			33.3		0.0	0.0	100.0	50.0
Cephalexin	52.6			10.5	100.0				
Clindamycin				50.0					
Erythromycin	31.7			7.9	47.1		40.0	100.0	
Ceftriaxone	45.3			9.7	73.5	50.0	50.0		66.7
Cefepime				0.0	75.0	100.0			
Azithromycin									75.0
Amoxicillin						100.0	100.0		
Norfloxacin							100.0		

Abbreviations: CoNS, coagulase negative *Staphylococcus spp.*; GPC, gram-positive Cocci; HLAR, high-level aminoglycoside resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.

spp. isolates carried genes for HLAR, where a combination of β-lactams and aminoglycosides may not work in vivo for therapy even though they may have been sensitive in vitro. Coagulase negative *Staphylococcus* (CoNS), long considered as contaminants in 1970s and 1980s are nowadays considered to be agents capable of causing bacteremia. They are mostly incriminated as nosocomial pathogens specifically in catheter-related BSI. In fact, two studies[17,28] reported CoNS as the most common isolate causing BSIs in ICU patients. Seven such isolates of CoNS were recovered in the present study which fulfilled the criteria for sepsis.

All patients with fungemia were critical and under observation in different ICUs. Risk factors for fungemia include prolonged hospital stay, hyper alimentation, previous broad-spectrum antimicrobial therapy, and ulcerations in gastrointestinal mucosa. Prognosis in fungemia patients is relatively poor.[29]

Frequency of isolation on different days of incubation within the 7-day incubation period reveals interesting trends. Most of the prominent isolates had a peak of detection on the second day of incubation which gradually tapered within the next 2 days. However, there was no significant difference observed between organisms isolated within the first 48 hours or beyond initial 48 hours.

An attempt was made to evaluate any correlation between the source of blood collection and the frequency of organisms isolated. The data for this information were available roughly for approximately 3 years (2011–2013) only. Chances of recovery of positive blood culture isolates from central line catheters were significantly higher in *A. baumannii* ($p < 0.0029$) and *K. pneumoniae* ($p < 0.002$). This is easily comprehensible since central venous catheters are kept in situ for longer durations compared with peripherally inserted catheters. These organisms are known to produce biofilm which improves their persistence within intravascular devices. Biofilm formation also imparts them the ability to reduce their metabolism rate, prevent antibiotic entry, and promote transfer of resistance plasmids.

Table 7 Drug sensitivity profile of gram-negative bacilli strains

Organism	Ceftriaxone	Ceftazidime	Cefoperazone sulbactam	Cefoperazone	Cefotaxime	Piperacillin	Piperacillin-tazobactam	Imipenem	Meropenem	Levofloxacin	Ciprofloxacin	Ofloxacin	Ampicillin	Gentamicin	Amikacin	Chloramphenicol	Colistin	Cefepime
Overall GNB	23.8	19.2	83.1	38.3	26.4	30.7	69.6	86.9	89.1	68.7	48.6	59.3	10.8	58.0	63.0			19.4
Acinetobacter baumannii	19.6	12.6	79.1		27.7	35.3	62.8	78.4	100.0	65.7	58.6	60.8		64.9	43.8	45.5	16.7	30.8
Acinetobacter lwoffii	20.0	23.5	66.7	100.0	25.0	26.7	50.0	68.2	66.7	77.8	63.6	87.5		76.5	71.4	66.7		
Escherichia coli	20.0	21.9	92.0		11.8	17.2	79.3	100.0	100.0	16.7	15.4	20.0	9.4	86.2	85.7	100.0		
Klebsiella pneumoniae	10.6	7.2	81.6	0.0	14.1	9.7	73.6	98.4	93.8	66.7	39.7	59.1	2.9	55.0	61.5	76.0	40.0	12.5
Klebsiella oxytoca	0.0	0.0	100.0			0.0	60.0	100.0		75.0		66.7	0.0	0.0	50.0	50.0		
Pseudomonas aeruginosa	23.3	28.8	80.8	44.8	50.0	52.8	68.9	83.9	63.6	71.4	37.2	40.0		34.0	65.0		44.4	22.2
Nonfermenter GNB	50.0	56.0	96.2	25.0	69.2	66.7	88.9	66.7	87.5	87.5	86.4	85.2	37.9	73.1		100.0	100.0	0.0
Enterobacter spp.	30.0	18.2	83.3		25.0	27.8	72.0	96.0	83.3	83.3	66.7	78.9	4.8	30.4	66.7	20.0	100.0	0.0
Citrobacter spp.		28.6	100.0		0.0	42.9	42.9	100.0			40.0	50.0		50.0	50.0	50.0		0.0
Proteus spp.	25.0	0.0	100.0			20.0	20.0	20.0			80.0	80.0		75.0				
Morganella morganii		0.0	100.0		0.0	0.0	100.0	100.0			0.0	0.0		50.0				
Haemophilus influenzae					100.0	100.0				100.0			100.0			100.0		100.0
Salmonella spp.	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	88.9			100.0		

Abbreviation: GNB, gram-negative Bacilli.

Such foci of bacteria can lead to sustained bacteremia if not dislodged in time, or catheter being removed. Contamination rates were similar for both sources of blood collection at approximately 1%. A study by Beutz et al[30] claims sensitivity of 92.5 and 95.9% for blood cultures drawn from central vein catheters and peripheral venipuncture, respectively. Even though the negative predictive values of both sources of blood collection was > 95%, positive predictive values of blood cultures were low at 58.3 and 66.7%, respectively.

Beta lactam drugs are rapidly becoming ineffective for treating BSIs because of its indiscriminate and nonjudicious usage. Vancomycin, linezolid, aminoglycosides, and broad-spectrum drugs like chloramphenicol and tetracycline are the most reliable treatment options for GPCs, whereas carbapenems, CFS, aminoglycosides, and quinolones are remaining treatment options for GNBs. Aminoglycosides are good options for *E. coli* but not *K. pneumoniae* BSIs, while the opposite is true for quinolones. Cephalosporins and typical antipseudomonal drugs failed terribly in controlling concerned BSIs. Hence rationalized drug therapy is the call of the hour and therefore, studies of this type are quite warranted. De-escalation of high-end antimicrobials once actual sensitivity pattern is known contributes to reduction of antimicrobial pressure. Poor infection control practices and inappropriate use of antibiotics are main driving forces for the spread of resistant organisms. Aggressive measures like routine surveillance cultures to identify and isolate carriers, control of environmental sources, antibiotic restriction, antibiotic recycling, recommending combination therapy, implementing proper aseptic techniques, performing hand hygiene, maintaining robust infection control practices, and periodical assessment of antimicrobial policy will go a long way in preventing emergence of resistant organisms.

Conclusion

Successful treatment of sepsis cases hinges on early diagnosis and proper antimicrobial therapy. The choice of antibiotics is based upon local knowledge of bacteriological profile and antimicrobial sensitivity patterns. The serious nature of the BSIs underscores the importance of periodic epidemiological surveillance studies such as the current one to provide useful insights for rational policy development and management of similar infections.

Financial Support
None.

Ethical Approval
Approved.

Informed Consent
Retrospective observational analysis, consent could not be obtained.

Conflict of Interest
None declared.

Acknowledgment
The authors would like to thank Mr. Pandurang V. Thatkar for technical and statistical help.

References

1 Seifert H, Wisplinghoff H, Bloodstream infection and endocarditis. In: Borriello SP, Murray PR, Funke G, eds. Topley and Wilson's Microbiology and Microbial Infections, Bacteriology Vol 1. 10th ed. London: Hodder Arnold ASM Press; 2005 1181–1235
2 Munford RS, Suffredini AF, Sepsis, severe sepsis and septic shock. In: Bennett JE, Dolin R, Blaser MJ, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases Vol 1. 8th ed. Philadelphia, PA: Elsevier Saunders; 2015 914–934
3 Vasudeva N, Nirwan PS, Shrivastava P. Bloodstream infections and antimicrobial sensitivity patterns in a tertiary care hospital of India. Ther Adv Infect Dis 2016;3(5):119–127
4 Weinstein MP. Current blood culture methods and systems: clinical concepts, technology, and interpretation of results. Clin Infect Dis 1996;23(1):40–46
5 CLSI, Principles and Procedures for Blood Cultures; Approved Guideline. CLSI Document M47-A.Wayne, PA: Clinical and Laboratory Standards Institute; 2007
6 Performance standards for Antimicrobial Susceptibility Testing, Twenty-Fourth Informational Supplement. CLSI Document M100–S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014
7 Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev 2006;19(4):788–802
8 Weinstein MP, Reller LB, Murphy JR, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev Infect Dis 1983;5(1):35–53
9 Weinstein MP. Blood culture contamination: persisting problems and partial progress. J Clin Microbiol 2003;41(6): 2275–2278
10 Prabhu K, Bhat S, Rao S. Bacteriologic profile and antibiogram of blood culture isolates in a pediatric care unit. J Lab Physicians 2010;2(2):85–88
11 Kollef MH, Sherman G, Ward S, Fraser VJ. Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. Chest 1999;115(2): 462–474
12 Leibovici L, Paul M, Poznanski O, et al. Monotherapy versus beta-lactam-aminoglycoside combination treatment for gram-negative bacteremia: a prospective, observational study. Antimicrob Agents Chemother 1997;41(5):1127–1133
13 Leibovici L, Shraga I, Drucker M, Konigsberger H, Samra Z, Pitlik SD. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. J Intern Med 1998;244(5):379–386
14 Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest 2000;118(1):146–155
15 Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34(6):1589–1596
16 Jagdish L, Naik TB, Gupta RK, Jais M. Etiology of blood culture from septicemia cases and their antibiotic susceptibility pattern at a tertiary care hospital. Indian J Microbiol Res 2016;3:436–440
17 Wattal C, Raveendran R, Goel N, Oberoi JK, Rao BK. Ecology of blood stream infection and antibiotic resistance in intensive

care unit at a tertiary care hospital in North India. Braz J Infect Dis 2014;18(3):245–251
18 Rajeevan S, Ahmed SM, Jasmin PT. Study of prevalence and antimicrobial susceptibility pattern in blood isolates from a tertiary care hospital in North Kerala, India. Int J Curr Microbiol Appl Sci 2014;3:655–662
19 Sharma R, Sharma R, Gupta S. Bacteriological analysis of blood culture isolates with their antibiogram from a tertiary care hospital. Int J Pharm Sci Res 2015;6:4847–4851
20 Gupta S, Kashyap B. Bacteriological profile and antibiogram of blood culture isolates from a tertiary care hospital of North India. Trop J Med Res 2016;19:94–99
21 Roy I, Jain A, Kumar M, Agarwal SK. Bacteriology of neonatal septicaemia in a tertiary care hospital of northern India. Indian J Med Microbiol 2002;20(3):156–159
22 Gohel K, Jojera A, Soni S, Gang S, Sabnis R, Desai M. Bacteriological profile and drug resistance patterns of blood culture isolates in a tertiary care nephrourology teaching institute. BioMed Res Int 2014;2014:153747
23 Tariq TM. Bacteriologic profile and antibiogram of blood culture isolates from a children's hospital in Kabul. J Coll Physicians Surg Pak 2014;24(6):396–399
24 Quereshi M, Aziz F. Prevalence of microbial isolates in blood cultures and their antimicrobial susceptibility profiles. Biomedica 2011;27:136–139
25 Meremikwu MM, Nwachukwu CE, Asuquo AE, Okebe JU, Utsalo SJ. Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. BMC Infect Dis 2005;5:110
26 Archibald LK, Pallangyo K, Kazembe P, Reller LB. Blood culture contamination in Tanzania, Malawi, and the United States: a microbiological tale of three cities. J Clin Microbiol 2006;44(12):4425–4429
27 Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. J Clin Microbiol 2003;41(8):3655–3660
28 Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF, Volturo GA. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Microbiol Antimicrob 2004;3:7
29 Bryan CS. Clinical implications of positive blood cultures. Clin Microbiol Rev 1989;2(4):329–353
30 Beutz M, Sherman G, Mayfield J, Fraser VJ, Kollef MH. Clinical utility of blood cultures drawn from central vein catheters and peripheral venipuncture in critically ill medical patients. Chest 2003;123(3):854–861