ORIGINAL RESEARCH

A Study of Pathogenic Bacteria Isolated from Blood Culture Samples and Their Antibiotic Sensitivity

[1]Tiwari Palak, [2]Mahi Ishani, [3]Lal Darbari

[1]Senior Resident, [2]Junior Resident, Department of Nursing Home (Medicine), Hindu Rao Hospital, Delhi, India
[3]Physician, Chief Medical Officer (SAG), Department of Medicine, Hindu Rao Hospital, Delhi, India

Correspondence:
Darbari Lal
Physician, Chief Medical Officer (SAG),Department of Medicine, Hindu Rao Hospital, Delhi, India
Email: dr_darbarilal@rediffmail.com

ABSTRACT

Background: To diagnose bacteremia blood culture still remains the gold standard despite its limitations. While collecting samples, focus must be given that there is strong association between timing of specimen collection at different time points during admission and their yield.

Methods: A retrospective observational study was carried out by analyzing 100 positive blood cultures from April 2021-April2022. All positive blood culture and sensitivity reports of males and females aged less than one month to 60years were included. A total of 100 positive blood culture cases were taken from the culture and sensitivity register from Microbiology department of Hindu Rao Hospital and details were tabulated using a questionnaire.

Results: The commonest organism found on cultures was Coagulase Positive Staphylococcus (CONS) 34% of the cultures, this was followed by Actinobacter in 17% and Klebsiella pneumoniae in 11% of the culture samples. The most common fungi were Candida in 3% and Budding Yeast which was found in 2% of the samples. The most sensitive antibiotic was Gentamycin (27) which was sensitive for Coagulase-positive SA (12), K. pneumoniae (5), Actinobacter (5), S. typhi (3), E. coli (1) and P. aeuroginosa (1). The second most sensitive antibiotic was Vancomycin (20), which was sensitive for Coagulase-positive SA (9), Enterococcus (4), Methicillin resistant coagulase-positive SA (2), Methicillin sensitive SA (2), Actinobacter (1), MRSA (1), and S. aureus (1).

Conclusion: Positive blood culture is a crucial parameter for both the diagnosis of the patientas well as the associated prognosis, the correct interpretation of the blood culture results is essential. While planning treatment the sensitivity and resistance pattern of pathogens found in blood culture to common antimicrobial agents must be taken into account.

Keywords: Antibiotic Sensitivity, Bacteremia, Blood Culture, Antibiotic Resistance.

INTRODUCTION

Establishing Bacteremia and fungemia have been one of the essential functions of clinical microbiology. When cultures reveal a clinically significant microorganism, it is suggesting the failure of the host defenses to contain an infection or the prescribed antimicrobials failing

to adequately eradicate the infectious process. The presence of bacteremia or fungemia also indicates disseminated infection and is generally associated with poorer prognosis than localized disease.[1]A positive blood culture, however, is not always clinically significant, as it may represent contamination due to improper technique or transient or self-limited presence of microorganisms in the blood.[1] Effective use of this function requires careful consideration of specimen collection and processing, culture techniques, result reporting, and, most importantly, result interpretation by the physician.[2] Knowledge of frequently encountered pathogens along with their antimicrobial sensitivity profile is invaluable in treating individual patients, as well as designing appropriate protocols.[3] This study was conducted to offer insight into the microbiological profile and antimicrobial susceptibility patterns in blood culture and sensitivity samples of patients of encountered at Hindu Rao Hospital, Delhi.

MATERIALS AND METHODS

This is a cross-sectional study conducted at Hindu Rao Medical College & Hospital. For a 12-month period, April-2021 through April-2022, all inpatients with culture-positive blood were evaluated at Hindu Rao Medical College and Hospital, Delhi. Prior to the study, all relevant permissions were obtained from the competent hospital authorities. Each patient was observed prospectively from the time the blood culture became positive, unless the patient had been discharged or had died before review of the hospital records by one of the investigators, in which case the review of the medical record was retrospective.[1] Charts were evaluated such that if the presence or absence of a particular finding was not clearly indicated, that case was excluded from analysis for that finding.[1,2]

During the study period, 20 mL or more of blood was obtained for each culture and inoculated into media for processing blood culture system.[2] Blood was obtained at the bedside by trained health care personnel (nurses, phlebotomists, etc.) using 70% isopropyl alcohol and then 10% povidone-iodine. Although majority of the blood culture specimens were obtained by peripheral venipuncture[4], it was not possible to determine specifically which ones were obtained in this manner and which were obtained through access devices.[1,4] Culture bottles were transported to the laboratory and incubated until flagged as positive or for 7 days. Broth from positive bottles was gram-stained and subcultured with use of standard techniques. Susceptibility testing of isolates was done according to guidelines established.[1] All information obtained from patients' records was recorded on data worksheets and analysis done using MS Excel.

PROCEDURE

1. Skin Antisepsis: Local antisepsis done with 70% isopropyl alcohol and then 10% povidone-iodine before obtaining sample.
2. Method of obtaining blood for culture: Venipuncture remains the method of preference for obtaining blood for culture; arterial blood is not associated with betterdiagnostic yields.[4,5]
3. Number of blood cultures: Single blood cultures should be discouraged as they are insufficiently sensitive for detecting some bacteremias and fungemias, but also may be difficult to interpret. For majority of patients, two blood cultures should be sufficient.[1,4]
4. Timing of blood cultures: 30-to-60-minute interval, except for critically ill, septic patients from whom specimens should be obtained minutes before initiating therapy.[1,5]
5. Volume of blood required: The recommended volume of blood per culture set for andadult is 10-30mL, and the preferred volume is 20-30mL. For infants and small children, 1-2mL of blood per culture for neonates, 2-3mL for infants aged 1month

to 2years, 3-5mL forolder children, and 10-20mL for adolescents.[1,6]

6. Culture Media: Most commercially available and microbiology lab prepared blood culture media perform well as long as cross contamination is avoided.
7. Blood-to-broth ratio: Blood should be diluted fivefold to tenfold. Dilutions of <1:5 and >1:10 may be associated with reduced yield.[1,6]
8. Atmosphere of incubation: Routine use of anaerobic blood culture bottles is not necessary and are to be used selectively for patients who are high risk for bacteremia due toanaerobes.
9. Length of incubation of blood cultures: In usual circumstances, blood cultures don't need to be incubated for >7 days. Incubation periods longer than 7 days may be useful when fungemia or bacteremia due to fastidious organisms such as HACEK group of bacteria or species of Legionella or Brucella are suspected. Mycobacterial blood cultures should be incubated for >4weeks.[1,5]

INTERPRETATION

Parameters that are helpful in the interpretation of results include the identity of the microorganism; the presence of the same microorganism as found in the blood from another normally sterile site.

A useful concept is the number of culture sets found to be positive vs. the number obtained. If most or all cultures in a series are positive, regardless of the microorganism recovered, the probability that the organism is clinically significant is high.[1]

RESULTS

Out of 100 positive blood culture the highest distribution was in the <1month age group with 25 in male and 17 in female. This was followed by the 16–30-year age group, with 7 females and 5 males having positive cultures. The 46–60-year age group had 5 males and 4 females showing growth on cultures.

The most common organism found on cultures was found to be Coagulase Positive Staphylococcus (CONS) which was found in 34% of the cultures, this was followed by Actinobacter in 17% and Klebsiella pneumoniae in 11% of the culture samples. The most common fungi were Candida in 3% and Budding Yeast which was found in 2% of the samples.

On further analysis of sensitivity, the most sensitive antibiotic was found to be Gentamycin(27) which was sensitive for Coagulase-positive SA (12), K. pneumoniae (5), Actinobacter(5), S. typhi (3), E. coli (1) and P. aeuroginosa (1). The second most sensitive antibiotic was Vancomycin (20), which was sensitive for Coagulase-positive SA (9), Enterococcus (4), Methicillin resistant coagulase-positive SA (2), Methicillin sensitive SA (2), Actinobacter (1), MRSA (1), and S. aureus (1). Followed by Linezolid (19) which was found to be sensitive for Coagulase-positive SA (8), MRSA (3), S. aureus (5), and Streptococcus (3).

Fig 1: Age and sex distribution of patients with positive blood culture

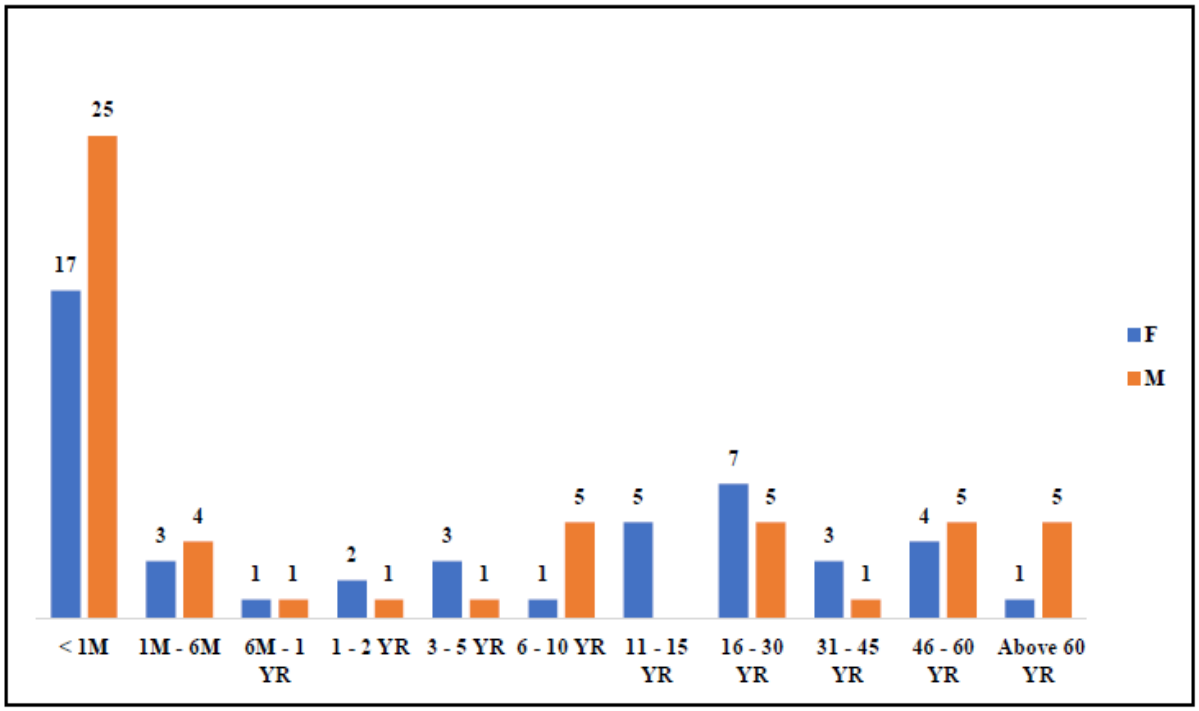

Fig 2: Antibiotic sensitivities of microorganisms (AK: Amikacin; AT: Aztreonam; C: Chloramphenicol; CIP: Ciprofloxacin; CTR: Ceftriaxone; G: Gentamycin; LE: Levofloxacin; LZ: Linezolid; TGC: Tigecycline; TIG: Tigecleror; V9: Vancomycin)

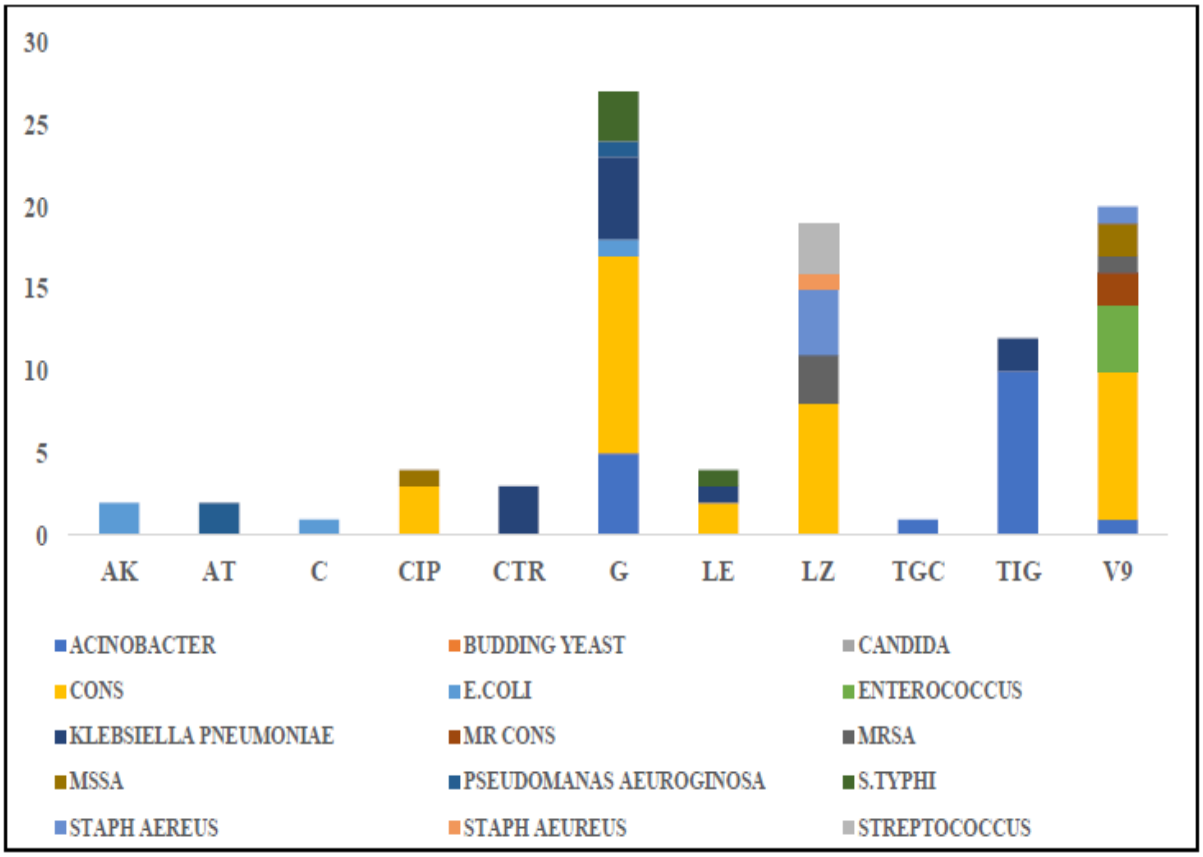

Fig 3: Klebsiella-MacConkey media

Fig 4: S. aureus-Blood agar

Fig 5: E. coli- MacConkey Media

Fig 6: Coagulase-Negative SA-Blood agar

Table 1: Distribution of Blood Culture Isolates (CoNS: Coagulase Negative Staphylococcus aureus; MR CoNS: Methicillin Resistant CoNS; MRSA: Methicillin Resistant Staphylococcus aureus; MSSA: Methicillin Sensitive Staphylococcus aureus)

INFECTION					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Acinobacter	17	17.0	17.0	17.0
	Budding Yeast	2	2.0	2.0	19.0
	Candida	3	3.0	3.0	22.0
	CONS	34	34.0	34.0	56.0
	E. Coli	4	4.0	4.0	60.0
	Enterococcus	4	4.0	4.0	64.0
	Klebsiella Pneumoniae	11	11.0	11.0	75.0
	MR Cons	2	2.0	2.0	77.0
	MRSA	4	4.0	4.0	81.0
	MSSA	3	3.0	3.0	84.0
	Pseudomonas Aeuroginosa	3	3.0	3.0	87.0
	S. Typhi	4	4.0	4.0	91.0
	Staph Aureus	5	5.0	5.0	96.0
	Staph Aureus	1	1.0	1.0	97.0
	Streptococcus	3	3.0	3.0	100.0
	Total	100	100.0	100.0	

Table 2: Distribution of Antibiotic Sensitivity

SENSITIVITY OF ANTIBIOTICS					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid		5	5.0	5.0	5.0
	AK	2	2.0	2.0	7.0
	AT	2	2.0	2.0	9.0
	C	1	1.0	1.0	10.0
	CIP	4	4.0	4.0	14.0
	CTR	3	3.0	3.0	17.0
	G	27	27.0	27.0	44.0
	LE	4	4.0	4.0	48.0
	LZ	19	19.0	19.0	67.0
	TGC	1	1.0	1.0	68.0
	TIG	12	12.0	12.0	80.0
	V	20	20.0	20.0	100.0
	Total	100	100.0	100.0	

DISCUSSION

In our study we evaluate 100 positive blood culture reported in our hospital during 12-month period, April-2021 through April-2022, mostly inpatients. The causative agents responsible for bloodstream infections differ from country to country with geographical variations [7, 8, 9]. Various studies done by Bouza et al.[10] in Spain, Koupetori et al.[11] in Greece, and Musicha et al.[12] in Malawi shows predominance of Gram-negative bacteria. However, other studies done by Kolonitsiou et al.[13] in Greece, Bassetti et al.[14] in Italy, and Wasihun et al.[15] in Ethiopia, as well as Chiduo et al.[16] in Tanzania proved Gram-positive bacteria to be predominantly responsible for bloodstream infections. In our study we observed most common organism Coagulase Positive Staphylococcus (CONS) which was found in 34% of

the cultures, this was followed by Actinobacter in 17% and Klebsiella pneumoniae in 11% of the culture samples. This contrary to the findings of Labi et al.[17], Obeng-Nkrumah et al.[18], and Opota et al.[19] who reported Escherichia coli as the leading cause for bloodstream infections. Bacteraemia was more among young children as compared to the older age groups. Among both male and female subpopulations an inverse relationship between age and the incidence of bloodstream infection was established. This finding suggests that young children are at a greater risk of acquiring bloodstream infections compared to the older age groups. Postulated factors attributed to higher bloodstream infection rate in young patients particularly neonates include immature immune system, poor skin integrity, frequent exposure to healthcare environments, and low socioeconomic status of parents, as well as poor hygiene practices, bottle feeding, and high incidence of delivery at home.[20-23] We observed that susceptibility to bloodstream infections is more in males. Thus, more males (53 %) recorded bacteraemia compared to their female counterparts (47%) (Fig. 1). these results add to a growing body of knowledge where male preponderance to bloodstream infections has been reported in the previous studies.[8,16,24-26] Among various reasons proposed to explain the male gender vulnerability include less frequent hand hygiene practice which could potentially provide enabling environment for large reservoirs of common pathogens responsible for causing bloodstream infections[27,28], biological makeup of women where oestrogen suppresses the expression of virulence factors of some microorganisms especially Pseudomonas aeruginosa.[27]

In our study most sensitive antibiotic was found to be Gentamycin (27) which was sensitive for Coagulase-positive SA (12), K. pneumoniae (5), Actinobacter (5), S. typhi (3), E. coli (1) and P. aeuroginosa (1). The second most sensitive antibiotic was Vancomycin (20), which was sensitive for Coagulase-positive SA (9), Enterococcus (4), Methicillin resistant coagulase-positive SA (2), Methicillin sensitive SA (2), Actinobacter (1), MRSA (1), and S. aureus (1). Followed by Linezolid (19) which was found to be sensitive for Coagulase-positive SA (8), MRSA (3), S. aureus (5), and Streptococcus (3).

CONCLUSION

The presence of a bloodstream infection is a crucial parameter for both the diagnosis of the patient as well as the associated prognosis, the correct interpretation of the blood culture results is essential. Updated knowledge of not just the commonly found microorganism in the bloodstream but their sensitivity profile can prove to be an invaluable weapon in a physician's arsenal. This would help impart quality healthcare to individual patients and also improve overall patient outcomes. However, the importance of correct interpretation and its corelation with patient condition cannot be stressed enough. The physician who must ultimately make the final judgement must take into account not only the laboratory findings but also the clinical presentation of the patient. Such studies, conducted on a larger scale and more frequently would also help local health authorities design better protocol and improve public messaging.

REFERENCES

1. Melvin P. Weinstein, Michael L. Towns, Seth M. Quartey, Stanley Mirrett, Larry G. Reimer, Giovanni Parmigiani, L. Barth Reller, The Clinical Significance of Positive Blood Cultures in the 1990s: A Prospective Comprehensive Evaluation of the Microbiology, Epidemiology, and Outcome of Bacteremia and Fungemia in Adults. Clinical Infectious Diseases 1997 April; 24(4): 584602.
2. Kirn, T. J., & Weinstein, M. P. Update on blood cultures: How to obtain, process,report, and interpret. Clinical Microbiology and Infection 2013; 19(6), 513-20.
3. Felices FJ, Hernandez JL, Ruiz J, Meseguer J, Gomez JA, Molina E. Use of the central

venous pressure catheter to obtain blood cultures. Crit Care Med 1979;7:78-9.
4. Tafuro P, Colbourn D, Gurevich I, et al. Comparison of blood cultures obtained simultaneously by venepuncture and from vascular lines. J Hosp Infect 1986;7:283-8.
5. Bryant JK, Strand CL. Reliability of blood cultures collected from intravascular catheter versus venipuncture. Am J Clin Pathol 1987;88:113-6.
6. Aronson MD, Bor D R Blood cultures. Ann Intern Med 1987; 106: 246-53. IsaacmanDJ, Karasic RE. Utility of collecting blood cultures through newly inserted intravenous catheters. Pediatr Infect Dis J 1990;9: 815-8.
7. H. Wisplinghoff, T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond, "Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study," Clinical Infectious Diseases 2004; 39(3): 309–17.
8. C. Akoua-Koffi, H. Tia, J. K. Plo et al., "Epidemiology of community-onset bloodstream infections in Bouaké, Central Côte d'Ivoire," New Microbes and New Infections2015; 7: 100–04.
9. D. J. Anderson, R. W. Moehring, R. Sloane et al., "Bloodstream infections in community hospitals in the 21st century: a multicenter cohort study," PLoS One 2014; 9(3):Article ID e91713.
10. C. Bouza, T. López-Cuadrado, Z. Saz-Parkinson, and J. M. Amate-Blanco, "Epidemiology and recent trends of severe sepsis in Spain: a nationwide population-based analysis (2006–2011)," BMC Infectious Diseases 2014; 14(1):3863.
11. M. Koupetori, T. Retsas, N. Antonakos et al., "Bloodstream infections and sepsis in Greece: over-time change of epidemiology and impact of de-escalation on final outcome," BMC Infectious Diseases 2014; 14(1): 272.
12. P. Musicha, J. E. Cornick, N. Bar-Zeev et al., "Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study," The Lancet Infectious Diseases 2017; 17(10): 1042–52.
13. F. Kolonitsiou, M. Papadimitriou-Olivgeris, A. Spiliopoulou et al., "Trends of bloodstream infections in a University Greek Hospital during a three-year period: incidenceof multidrug-resistant bacteria and seasonality in gram-negative predominance," Polish Journal of Microbiology 2017; 66(2): 171–80.
14. M. Bassetti, E. Righi, and A. Carnelutti, "Bloodstream infections in the intensive care unit," Virulence, vol. 7, no. 3, pp. 267–279, 2016.
15. G. Wasihun, L. N. Wlekidan, S. A. Gebremariam et al., "Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients in Mekelle hospital, Northern Ethiopia," Springer Plus 2015; 4(1): 314.
16. M. G. Chiduo, M. Kamugisha, A. Mhina et al., "Possible causes of fever among patients with blood smear negative for malaria parasites at Bombo regional referral hospital in Tanga, Tanzania," Tanzania Journal of Health Research 2017; 19(4).
17. A.-K. Labi, N. Obeng-Nkrumah, S. Bjerrum, C. Enweronu-Laryea, and M. J. Newman, "Neonatal bloodstream infections in a Ghanaian Tertiary Hospital: are the current antibiotic recommendations adequate?" BMC Infectious Diseases 2016; 16(1): 598.
18. N. Obeng-Nkrumah, A.-K. Labi, N. O. Addison, J. E. M. Labi, and G. Awuah- Mensah, "Trends in paediatric and adult bloodstream infections at a Ghanaian referral hospital: a retrospective study," Annals of Clinical Microbiology and Antimicrobials 2016;15(1): 49.
19. O. Opota, K. Jaton, and G. Greub, "Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood," Clinical Microbiology and Infection 2015; 21(4): 323–31.
20. M. Dagnew, G. Yismaw, M. Gizachew et al., "Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University

Hospital,Northwest Ethiopia," BMC Research Notes 2013; 6(1): 283.
21. Hojsak, H. Strizić, Z. Mišak et al., "Central venous catheter related sepsis in children on parenteral nutrition: a 21-year single-center experience," Clinical Nutrition 2012; 31(5): 672–75.
22. M. A. Ugas, H. Cho, G. M. Trilling et al., "Central and peripheral venous lines-associated blood stream infections in the critically ill surgical patients," Annals of SurgicalInnovation and Research 2012; 6(1): 8.
23. J. M. Costello, T. C. Clapper, and D. Wypij, "Minimizing complications associated with percutaneous central venous catheter placement in children," Pediatric Critical Care Medicine 2013; 14(3): 273–283.
24. Onken, A. K. Said, M. Jørstad, P. A. Jenum, and B. Blomberg, "Prevalence and antimicrobial resistance of microbes causing bloodstream infections in Unguja, Zanzibar," PLoS One, vol.10, no. 12, Article ID e0145632, 2015.
25. Mehl, B. O. Åsvold, S. Lydersen et al., "Burden of bloodstream infection in an area of Mid-Norway 2002–2013: a prospective population-based observational study," BMC Infectious Diseases 2017; 17(1): 205.
26. N. Buetti, J. Marschall, A. Atkinson, and A. Kronenberg, "National bloodstream infection surveillance in Switzerland 2008–2014: different patterns and trends for university and community hospitals," Infection Control & Hospital Epidemiology 2016; 37(9): 1060–67.
27. H. Humphreys, F. Fitzpatick, and B. J. Harvey, "Gender differences in rates of carriage and bloodstream infection caused by methicillin-resistant Staphylococcus aureus: arethey real, do they matter and why?" Clinical Infectious Diseases 2016; 61(11): 1708-14.
28. Garbutt, G. Simmons, D. Patrick, and T. Miller, "The public hand hygiene practices of New Zealanders: a national survey," The New Zealand Medical Journal (Online), 2007; 120(1265): 27–33.