International Journal of Medical and Allied Health Sciences

ISSN: 2583-1879
Available at http://www.ijmahs.org/

Original Research Article

Bacteriological Profile of Blood Culture in A Tertiary Care Hospital in Northern India

Shashi Kala Kharwar[1*] Shadma Yaqoob[2] and Sana Siddique[3]

[1]Dept. of Microbiology, Era's Lucknow Medical college and Hospital, Lucknow.
[2]Professor, Dept. of Microbiology, Era's Lucknow Medical college and Hospital Lucknow.
[3]Assistant Professor, Dept. of Microbiology, Era's Lucknow Medical college and Hospital, Lucknow.

Article Information: Received on 19-11-2022 Accepted 27-12-2022 Available online 29-12-2022

Abstract

Aim of the study: This study was carried out to find out the prevalence of bacteriological profile of blood culture in Northern India.
Material and Methods: - Present cross-sectional study was carried in the Department Microbiology, Era's Lucknow Medical college and Hospital, Lucknow. A total of 638 patients with clinically diagnosed bloodstream infections (BSIs) were included in the study using a standard sampling procedure. As the result, 638 blood culture samples from Era's Lucknow Medical College and Hospital, Lucknow, were collected and investigated in the Department of Medical Microbiology laboratory.
Inclusion criteria: All blood samples from patients with suspected bloodstream infections (BSI) who were admitted in the Tertiary Care Hospital were included.
Exclusion criteria: Blood samples from BSI patients with a history of antimicrobial therapy prior to sample collection were excluded.
Results: Among 638 sample, 90 (14.10%) samples were pathogenic. The gram-positive organism were 34 (37.78%) and gram-negative organism were 56(62.22%). In this study most commonly identified gram-negative bacteria were *Klebsiella species*. (31.11%), *Escherichia coli* (26.67%), *Pseudomonas species.* (3.33%), *Acinetobacter species.* (1.11%) and among gram-positive were Coagulase- Negative *Staphylococcus (CoNS)* (27.78%), *Enterococcus species* (6.67%), *Staphylococcus species.* (3.33%)
Conclusion: In our study *Klebsiella species* & *E. coli* among gram-negative bacteria, *CoNS* & *Staphylococcus aureus* among gram-positive were the predominant blood borne pathogens in all age group and both sexes.

Keywords: Blood stream infection (BSI), Bacteremia, Septicemia and Pathogenic Bacteria

1. Introduction

Bloodstream infections (BSIs) are the main cause of illness and mortality among hospitalized patients worldwide, affecting both sexes and all age groups [1]. The presence of pathogenic microorganisms causes an inflammatory response in the bloodstream, resulted in changes of patient's biochemical, clinical, and hemodynamic parameters [2]. Two major categories of bloodstream infections include Intravascular and extravascular.

* **Shashi Kala Kharwar**, Department of Microbiology, Era's Lucknow Medical college and Hospital, Lucknow - India,
E mail: shashikharwar8393@gmail.com
ORCID ID: 0000-0002-0000-0000
doi: https://doi.org/10.54618/IJMAHS.2022245

Presence of bacteria in blood is known as bacteremia that may be transient, continuous, or intermittent. With due course of time and due to some factors bacteremia further increases towards septicemia [3]. There are numerous bacteria that are associated with Bloodstream infections inclusive of Gram-positive bacteria: *Staphylococcus species, Streptococcus species* and *Enterococcus species* and Gram-negative bacteria: *Escherichia coli, Pseudomonas species, Klebsiella species, Salmonella species*. and *Enterobacter species* and [4,5].

2. Materials and Methods

Study Design –The Present study was a cross-sectional study with six months duration conducted at the Department of Microbiology of Era's Medical College and Hospital, Lucknow, U.P.

Inclusion Criteria – All blood sample of suspicious Bloodstream infection with patients admitted in the tertiary care hospital.

Exclusion Criteria - Blood samples of BSI patients with a history of antibiotic therapy prior to sample collection was not included.

Study population-Patients with high grade fever admitted in Era's Lucknow Medical college and Hospital, Lucknow.

Study population and sampling method- A total of 638 clinically diagnosed BSI patients were enrolled in the study using a standard sampling technique, which resulted in the collection of 638 blood culture samples from Era's Lucknow Medical College and Hospital, Lucknow, for analysis at the Department of Medical Microbiology laboratory.

Study procedure- Before beginning antimicrobial medication, aseptic blood samples were taken from each patient. Adults received 5–10 ml (average 7 ml), while children received 2–5 ml (average 3 ml) of inoculum in BacT/ALERT® FA and PF plus-aerobic bottles from bioMerieux, Durham. 40. In brief, these bottles were immediately incubated in the BacT/ALERT® 3D system (bioMerieux, Durham) after inoculation to detect aerobic growth in blood samples. A maximum of 7 days were allowed for the blood samples to be cultured.

If there was no growth, the outcome was seen negatively. A warning was automatically displayed by the BacT/ALERT® system (bio-Merieux, Durham) in the event of positive growth. Then, positive blood culture bottles were removed and sub-cultured on blood agar and MacConkey agar plates [6]. Plates inoculated blood agar and MacConkey agar were incubated for 18 to 24 hours at 37°C. Colony morphology, gram staining, and traditional biochemical testing were used in accordance with standard laboratory procedures to identify any bacterial growth on agar plates [7]. After collection of samples, the culture bottle was transferred to Department of Microbiology for incubation and subsequent processing. The inoculated BHI broth was incubated for 24hr under aerobic condition at 37°C.

Fig.-1 Showing BacT/ALERT® FA and PF plus-aerobic bottles

3. Results

3.1 Identification of bacteria

The organisms were identified by the characteristic colony morphology, pigment production & sugar fermentation (lactose fermenter & non -lactose fermenter), Odour, Gram staining, oxidase test and motility test by hanging drop method, and biochemical tests. Gram-positive cocci (GPC) were classified as either *Staphylococci* or *Streptococci/Enterococci species* based on Gram staining and catalase reactions. All of the GPC were divided into various species using coagulase and hemolytic responses on blood agar. The oxiod CM0888 Bile Esculin Agar was used to identify the different *Enterococcus species.*

3.2 Isolation

All clinical samples received in bacteriological laboratory were cultured on Nutrient agar, Blood agar and MacConkey agar. The plates were incubated for 14-28 hr. at 37 °C.

3.3 Colony characteristics of gram-positive isolates - Colony characteristics of Staphylococcus aureus on Nutrient agar plates size 1-4 mm in diameter with smooth, round, opaque and easily emulsifiable. Golden yellow non-diffusible pigments (Fig-2).

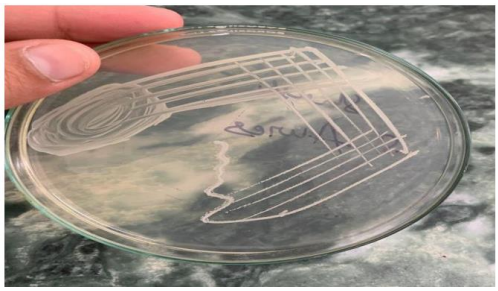

Fig. -2 The golden yellow pigment of *Staphylococcus species* shows on nutrient agar.

3.4 Colony characteristics of Staphylococcus on blood agar plates

In addition to being surrounded by a narrow zone of beta hemolysis on blood agar, colonies were identical to those on that nutrient agar (Fig-3).

Fig.-3 Blood agar-narrow zone of beta hemolysis of *Staphylococcus species.*

3.5 Colony characteristics of klebsiella on MacConkey agar plates

Due to the lactose fermentation, colonies were large, mucoid, dome shaped and sticky pink in color (Fig-4).

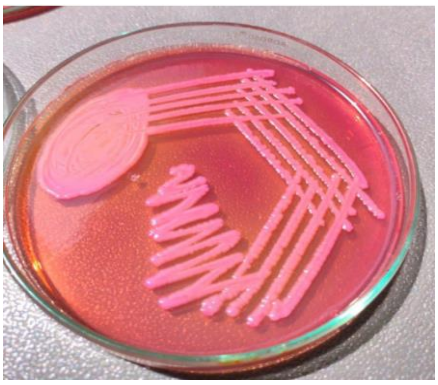

Fig -4 *Klebsiella spices* on MacConkey agar (lactose fermenting colonies, mucoid, dome shaped)

Identification was done using Gram stain, Biochemical tests, Antibiotic sensitivity test

Gram Staining- Mackie and McCartney; practical medical microbiology 14th edition – 2021): 45:796
Gram staining is one of the special and differential staining of microbiology, discovered by Danish Bacteriologist Hans Christian Gram (1882) who identified by causing pneumonia.

Coagulase test-By emulsifying a few pure *Staphylococcus* colonies from blood agar on undiluted human or rabbit plasma, the slide coagulase test of all isolates was carried out. Clump formation indicated, presence of *Staphylococcus aureus* in blood sample (Fig-5).

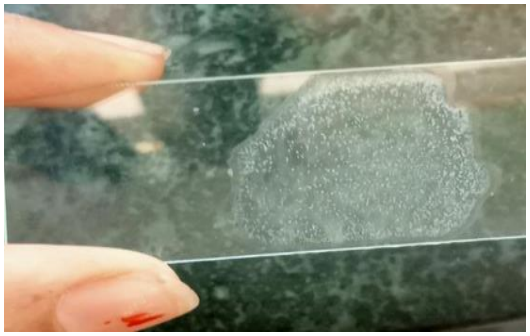

Fig.-5 Showing coagulase positive test

Bile-Esculin Hydrolysis test- One or two colonies were selected from an 18–24-hour culture using a sterile loop. with an S-shaped motion, injected onto the slope of the bile esculin medium. The inoculation tube was kept at 35 to 37°C for 24 hours of incubation. observed that more than half of the agar slant was dark brown or black in colour. show that an *Enterococcus species* (Fig-6).

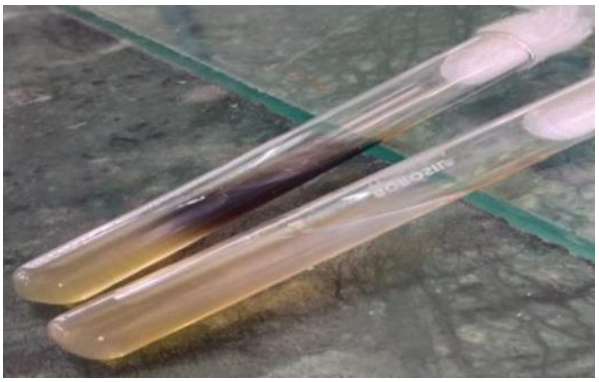

Fig.-6 Showing Bile-Esculin Hydrolysis positive test

Biochemical Tests: Different tests like Indole, MR, Urease, Citrate and Triple Sugar Iron (TSI) were done. TSI test provided a low degree of H_2S. Heavy inoculum was streaked over the surface of slope and stabbed into the butt, incubate aerobically at 37^0c for 24 hr., If sugar was fermented and Gas was produced, the reaction was positive. (A/A) by *Klebsiella species.* But not H_2S was produced (Fig-7).

Fig.-7 Showing Indole, MR, Urease, Citrate, TSI test

The present study was conducted in the Department of Microbiology at Era's Lucknow Medical College and Hospital. During the period from Dec 2022 to May 2023, 638 Blood samples from suspected septicemia patients were received and processed routinely.

Out of 638 blood cultures, 90 (14.10%) were positive for culture growth. (table-1).

Table -01 Percentage distribution of positive samples

Bacterial isolates	Numbers	Percentage
Gram positive	**34**	**37.78%**
Gram negative	**56**	**62.22%**
Total	**90**	**100%**

Among 638 sample, 90 (14.10%) samples were pathogenic. The gram-positive bacteria were 34 (37.78%) and gram-negative bacteria were 56 (62.22%). In this study most commonly, identified isolates were gram-negative bacteria (Fig.- 8).

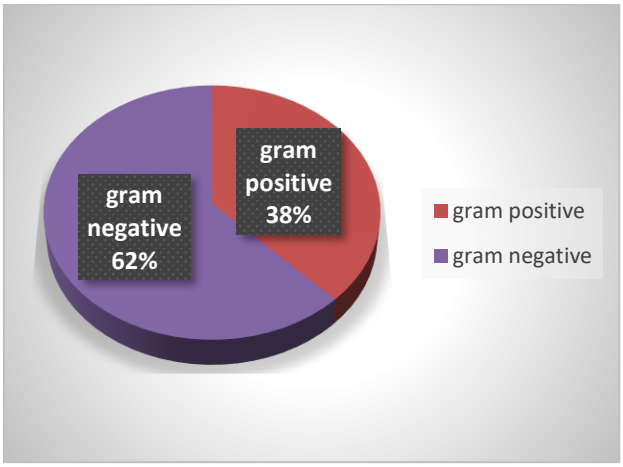

Fig.- -8 Showing percentage distribution of positive samples

Table - 2 Percentage of isolates found in blood samples

Bacterial isolates	Numbers	Percentage (%)
Klebsiella species	28	31.11%
Coagulase-negative staphylococcus (cons)	25	27.78%
E. Coli	24	26.67%
Enterococcus species	06	6.67%
Pseudomonas species	03	3.33%
Staphylococcus aureus.	03	3.33%
Acinetobacter species	01	1.11%
TOTAL	90	100%

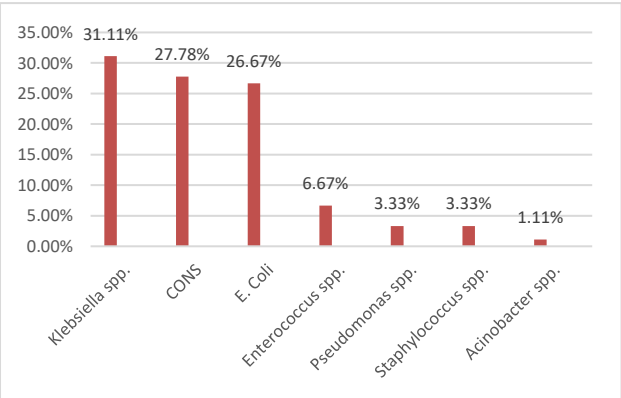

Fig. – 9 Showing percentage of isolates found in blood samples

Among 638 sample, 90 (14.10%) samples were pathogenic. The gram-positive bacteria were 34 (37.78%) and gram-negative bacteria were 56(62.22%). In this study most commonly identified gram-negative bacteria were *Klebsiella species*. (31.11%), *Escherichia coli* (26.67%), *Pseudomonas species.* (3.33%), *Acinetobacter species.* (1.11%) and among gram-positive were *Coagulase- negative Staphylococcus (CoNS)* (27.78%), *Enterococcus species* (6.67%), *Staphylococcus species.* (3.33%) (Fig.-9).

The most important task of a diagnostic microbiology laboratory is to immediately detect, identify, and test the antimicrobial susceptibility of bloodstream infection (BSI) pathogens, as they can be dangerous and difficult to treat [7, 8]. An average 8% reduction in survival occurs for every hour that therapy is delayed from starting. [9,10].

Culture positivity in the present study was 14.10%. This rate of isolation was supported by numerous studies conducted both domestically in India [11–13] and abroad [14, 15]. Some authors also showed a more positive culture [16, 17].

The current study conducted with the aim 'To study Bacteriological profile of blood culture & its drug resistance pattern in various wards of Era's Medical College and Hospital'. A total of 638 samples were collected in this cross-sectional study, included, out of 638 only 90 suspected were revealed to be septiceamic positive.

Among 90 isolates in the study, [18] (61.11%) were Gram-negative bacilli, Gram-negative cocci 1(1.11%) and 34 (37.78%) were gram-positive cocci, with *Coagulase-Negative Staphylococcus [CoNS*] being the most commonly isolated form. Out of 56 gram -negative bacilli, 28 (31.11%) isolates were *Klebsiella species,* 24 (26.67%) *Escherichia coli,* 3 (3.33%) *Pseudomonas species,* 1 (1.11%) *Acinetobacter species* and gram-positive cocci isolates were 25 (27.78%) *Coagulase-Negative Staphylococcus (CoNS),* 6 (6.67%) *Enterococcus,* 3(3.33%). *Staphylococcus species.* In the study of Ejaz *et al*, (2020) [19]. 50% were gram-negative rod and 49% gram-positive cocci followed by 1% candida. In other study by Muktikesh Das *et al,* (2016) [20]. 48.8% were gram -negative rod, 43.9% were gram-positive cocci and 7.3% were yeast. In comparison, Jyoti P. Sonawane *et al,* detected a higher percentage of gram-negative microorganism (lactose-fermenter & non-lactose fermenter) i.e..83.23% among blood culture isolates. In the present study, Coagulase-Negative Staphylococcus was the most commonly detected gram-positive bacteria (27,78%). most commonly isolated gram-positive bacteria was *Coagulase- Negative Staphylococcus* (27,78%), *Staphylococcus* are pathogens of man and other mammals and is listed in section 12 vol. 2 of Bergey's manual of systematic Bacteriology [21]. In certain situations, the typical skin commensals are increasingly considered bloodstream bacteria. The most frequent sources of bloodstream infections acquired in hospitals, as well as the most common blood contaminants, are improper blood collection techniques and the presence of long-standing intravascular catheters. [22].

Conclusion

- The current study was conducted on patients suffering from, Bloodstream infection, admitted in Era's Medical College and Hospital, Lucknow. A total of 638 blood samples were included in this study, although only 90 of them were considered to be septicemia-positive.

- A major public health problem that leads to high morbidity and mortality is bloodstream infections (BSIs). Effective diagnosis and treatment helped reduce mortality and morbidity. Effective diagnosis and treatment helped reduce mortality and morbidity.
- 90 samples (14.10%) out of the 638 found to be pathogenic. 34 (37.78%) of the bacteria were gram-positive, and 56 (62.22%) were gram-negative. In this study most commonly identified pathogenic organism were gram-negative bacteria.
- In our study *Klebsiella species* & *E. coli* among gram-negative bacteria, *CoNS* & *Staphylococcus aureus* among gram-positive were the predominant blood borne pathogens in all age group and both sexes.

Conflict of interest: Author declares that there is no conflict of interest.

References

[1]. Diekema DJ, Beekmann SE, Chapin KC, MorelKA, Munson E, Doern GV, Epidemelogy and outcome of nosocomial and community-onset bloodstream infection. *J Clin Microbial* 2003; 41:3655:60.

[2]. Claudio Viscoli (2016) Bloodstream Infections: The peak of the iceberg, Division of Infection Diseases, University of Genova (DISSAL), IRCCS San Martino-IST, Genova, Italy 5 February 2016 Virulence, VOL-7, Page no-3, 248-251, DOI: 10.1080/21505594.2016.1152440.

[3]. Goldstein E: Relation between prescribing of different antibiotics and rates of bacteremia/septicemia and associated mortality in the US and England (PREPRINT). *Bio Rxiv*. 2019, 10.1101/5.

[4]. Ahmed D, Nahid MA, Sami AB, et al. Bacterial aetiology of bloodstream infections and antimicrobial resistance in Dhaka, Bangladesh, 2005–2014. Anti-microbes Resist Infect Control. 2017; 6:2. https://doi.org/ 10.1186/s13756-016-0162-z.

[5]. Ndugulile F, Jureen R, Harthug S, Urassa W, Langeland N. Extended spectrum β-lactamases among gram-negative bacteria of nosocomial origin from an intensive care unit of a tertiary health facility in Tanzania. BMC Infect Dis. 2005; 5:86. https://doi.org/10.1186/1471-2334-5-86.

[6]. VITEK® 2 Automated Instrument for ID/AST Testing. Available from: http:// www. biomerieux- diagnostics.com/ vitek-2. [Last accessed on 2016 Apr 13].

[7]. Collee, J.G., Miles, R.S. and Watt, B. Tests for the Identification of Bacteria. In: Collee, J.G., Marmion, B.P., Fraser, A.G. and Simmons, A., Eds., Mackie & McCartney Practical Medical Microbiology, 14th Edition, Churchill Livingstone, New York, 1996; p. 131–151

[8]. Banik A, Bhat SH, Kumar A, Palit A, Snehaa K. Bloodstream infections and trend of antimicrobial sensitivity patterns at Port Blair. *J Lab Physicians* 2018; 10:332-7.

[9]. Vasudeva N, Nirwan PS, Shrivastava P. Bloodstream infections and antimicrobial sensitivity patterns in a tertiary care hospital of India. The Adv Infect Dis 2016;3: 119-27.

[10]. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, 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; 34:1589-96.

[11]. 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; 18:245-51.

[12]. 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; 19:94-9.

[13]. Tariq TM. Bacteriologic profile and antibiogram of blood culture isolates from a children's hospital in Kabul. *J Coll Physicians* Surg Pak 2014; 24:396-9.

[14]. Iregbu KC, Elegba OY, Babaniyi IB. Bacteriological profile of neonatal septicaemia in a tertiary hospital in Nigeria. Afr Health Sci 2006; 6:151-4.

[15]. Mehdinejad M, Khosravi AD, Morvaridi A. Study of prevalence and antimicrobial susceptibility pattern of bacteria isolated from blood cultures. *J Bio Science* 2009; 9:249-53[52] 15.

[16]. Sharma M, Goel N, Chaudhary U, Aggarwal R, Arora DR. Bacteraemia in children. *Indian J Pediatric* 2002; 69:1029-321

[17]. Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbial Rev 2006; 19:788-802.

[18]. Munford R. Sepsis, severe sepsis and shock. In: Mandell GL, Bennet JE, Dolin R, editors. Mandell, Douglas and Bennett's Principles and Practices of Infectious Diseases. 6th ed. London: Elsevier: Churchill Livingstone; 2005. p. 906-18.

[19]. Ejaz A, Khawaja A, Arshad F, et al. (October 17, 2020) Etiological Profile and Antimicrobial Patterns in Blood Culture Specimens in a Tertiary Care Setting. Cureus 12(10): e11000.

[20]. Dash M, Panda RK, Mohapatra D, Paty BP, Sarangi G, Chayani N. Bacteriological profile and antimicrobial resistance patterns of bloodstream infections in a tertiary care hospital, Eastern India. *Int J Health Allied Sci* 2016; 5:210-4.

[21]. Bhagat Singh and Renu Singh, Microbiology for Medical Sciences, Staphylococcus Infections., I. K. International Publishing House Pvt. Ltd. New Delhi, 2011, pp-145-152.

[22]. Vidyasagar K, Venkatesha D: Study of microbiological profile and antibiotic susceptibility of blood stream infections in tertiary care hospital. *Int J Current Microbial* App Sci. 2019, 8:1201-1211. 10.20546/ ijcmas.2019.807.143.