

Study of Central line related blood stream infection in intensive care units and its role in Biofilm formation: A Microbial Perspective

Dr. Dharmendra Singh, Dr. Suraiya Khanam Ansari, Dr. Vinay Shekhar,
Dr. Nashra Afaq, Dr. Sachin Kishore*

Department of Microbiology¹, G.S.V.M. Medical College, Kanpur, Uttar Pradesh, India.

Department of Microbiology², G.S.V.M. Medical College, Kanpur, Uttar Pradesh, India.

Department of Microbiology³, Maa Vindhyavasini Autonomous State Medical College, Mirzapur, India.

Department of Microbiology⁴, Rama Medical College Hospital & Research Centre, Kanpur, Uttar Pradesh, India.

Department of Microbiology*, Maa Vindhyavasini Autonomous State Medical College, Mirzapur, India.

Corresponding author : Dr. Sachin Kishore*
Email: sachinrastogi7819@gmail.com

KEYWORDS

Central line-related bloodstream infections (CLABSIs) Coagulase negative Staphylococcus (CONS), Antibiotic sensitivity, Biofilm production, Tube method.

ABSTRACT

Introduction: Central line-related bloodstream infections (CLRBSIs) are a significant cause of morbidity and mortality in intensive care units (ICUs). The development of biofilms on central venous catheters (CVCs) serves as a critical factor in the pathogenesis of these infections. **Aims and Objectives:** 1. To investigate the prevalence, microbial profile of central line-related bloodstream infections (CLRBSIs) in intensive care units (ICUs) and to explore the relationship between biofilm formation, antimicrobial resistance. 2. To determine the rate of central line related blood stream infection (CLRBSI). **Materials and Methods:** This prospective observational study was conducted in the ICU of a tertiary care hospital. Patients with suspected CLRBSIs were enrolled, and central venous catheter tips were collected aseptically for microbiological analysis. **Results & Conclusion:** CLRBSI was diagnosed in (32/150) patients and the rate of CVC- Blood stream infection number of CVC days was calculated as 17.5 per 1000 catheter days. The mean age of cases was observed to be 51 yrs. Patients from surgical ICU; signs of inflammation around catheter site, length of ICU stay, having underlying co-morbid conditions were significantly associated with CLRBSI. Gram positive organisms was the most common isolate. In our study duration of catheter more than seven days was associated with higher colonization rate. Hence regular surveillance for infection associated with them is essential.

INTRODUCTION

Different types of central venous catheters (CVCs) have been used in clinical practice to improve the quality of life of chronically and critically ill patients

Use of vascular catheters is common in both inpatient and outpatient care. CVCs play an integral role in modern Healthcare, their use however is associated with a risk of bloodstream infections caused by microorganism colonizing the external surface of device or the fluid pathway when the device is inserted or in course of its use [1] Gram-positive cocci are responsible for at least two-thirds of the infections followed by Gram-negative bacilli, which are responsible for a higher proportion of catheter related infections (CRIs) in intensive care unit (ICU) than in non-ICU patients.[2]

When microbes form a matured biofilm within human hosts through medical devices such as CVCs, the infection becomes resistant to antibiotic treatment and can develop into a chronic condition. For that reason, many techniques have been used to prevent the formation of biofilm by targeting different stages of biofilm maturation.

Biofilm is a fundamental component in the pathogenesis of infections related to the use of the central venous catheter (CVC,) which can represent an important health issue in everyday practice of nursing and medical staff.

Biofilm formation in catheters has not only been implicated as an important factor involved in device related infection but also confers resistance to antimicrobial treatment.[3] Staphylococcus aureus continue to be the most frequently encountered pathogens in device related infections. Other commonly encountered isolates include Enterococcus spp., CoNS, Pseudomonas aeruginosa, Klebsiella spp., Citrobacter spp., Acinetobacter baumannii, Candida albicans, Candida tropicalis etc.

OBJECTIVE OF STUDY

- To investigate the prevalence and microbial profile of central line-related bloodstream infections (CLRBSIs) in intensive care units (ICUs).
- To determine the rate of central line related blood stream infection (CLRBSI).
- To determine biofilm forming microorganisms that colonise the tip of catheter and their Resistance pattern.

MATERIAL & METHOD

The present prospective study was undertaken in the Department of Microbiology, GSVM Medical College Kanpur. The study was finally conducted on 150 patients admitted in medical and surgical ICU of the teaching hospital. Each patient's age, gender, clinical diagnosis, CVC insertion site and duration of duration of CVC catheterization, treatment given, duration of ICU stay; hospitalization and clinical outcome were duly recorded.

In present study, we follow Manual conventional blood culture method in which simultaneous blood sample (one from central line and one from peripheral veins) collected through the patients and inoculated on blood culture bottles and incubated at 37°C for 24 to 72 hr. Few drops from positive blood culture bottles were sub cultured on MacConkey agar, Nutrient agar and Blood agar plates.[4, 5]

Central lines were processed by using Semiquantitative Extraluminal Maki's roll over method. In this method Central lines were removed under strict Aseptic precaution Using sterile forceps, distal segment of central line was removed and catheter tip was rolled back and forth across agar surface using slight pressure at least four times with the help of sterile forceps. It was made sure

that the catheter tip has good contact with the surface of the Blood agar and MacConkey agar plate. The plates were incubated aerobically at 37°C for 48- 72hr. & colonies counted. [6, 7]The results were expressed as CFU. Significant growth is defined as ≥ 15 colony forming units (CFU). [8]

The organisms were identified by colony morphology, gram staining, and biochemical tests performed by routine laboratory techniques. All the isolates after identification were tested for their antibiotic susceptibility by using Kirby-Bauer disc diffusion method on Muller Hinton agar (MHA) plates and interpreted according to CLSI (Clinical and Laboratory Standards Institute), guidelines (M100, Ed33-March 2023). [9]

Biofilm production by Congo red agar method (CRA) Method

The medium composed of Brain heart infusion broth (37 mg/l), sucrose (5 mg/l), agar number 1 (10 mg/l) and Congo red dye (0.8 mg/l). Congo red stain was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes. Then it was added to autoclaved Brain heart infusion agar with sucrose at 55°C. Plates were inoculated with test organism and incubated at 37°C for 24 to 48 hours aerobically. Slime-producing strains form black colonies, whereas non producing strains develop pink colonies [10]

Inclusion criteria-

- Patients admitted in ICU, age more than 18 years.
- Patients developing systemic signs and symptoms >48 hrs. Of Central line insertion.
- Central line-related bloodstream infections (CLABSIs) are considered if positive blood culture with the same microorganism present in the tip of the catheter.

Exclusion criteria-

- Patients developing systemic signs and symptoms < 48 hrs. Of Central line insertion.
- Immunocompromised patients Receiving ART.
- Patients with obvious source of infection (fever, pneumonia, urinary tract infection, infective endocarditis and cellulitis) other than central venous catheter by history, clinical examination, etc.

The CLRBSI rate was to be calculated by the following formula [11]

CLRBSI rate per 1000 catheter days-

$$= \frac{\text{Number of CLRBSI cases} \times 1000}{\text{No. of CVC days}}$$

RESULT

The present study was conducted in a total of 150 patients. CLRBSI was diagnosed in 32 (21.33%) patients and the rate of CVC- Blood stream infection number of CVC days was calculated as 17.5 per 1000 catheter days. The mean age of cases was observed to be 51 yrs.

Table:1 Culture profile of Microorganisms causing CLRBSI (N=32)

Microorganisms	No. of isolates	%
S aureus	13	41%
CONS	9	28%
Escherichia coli	5	16%
Klebsiella pneumoniae	3	9%
Pseudomonas aeruginosa	2	6%
Total	32	100%

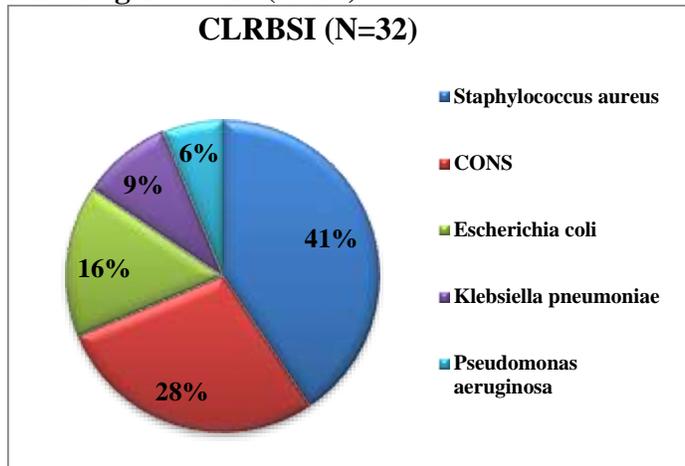


Table: 2 Organisms tested for biofilm by Congo red agar method

Organisms tested for biofilm	Congo red agar method		
	S	M	W/N
S aureus (n=13)	4 31%	3 23%	6 46%
CONS (n=09)	2 22%	5 55%	2 22%
E coli (n=05)	2 40%	1 20%	2 40%
K pneumoniae (n=03)	1 33%	1 33%	1 33%
P aeruginosa (n=02)	1 50%	0 0%	1 50%



Figure: 1 Detection of biofilm production by Congo Red Agar method black colonies of biofilm producer Staphylococcus aureus.

S=Strong M=Moderate W=Weak

Figure:2: Antibiotic Sensitivity Pattern of Gram Positive Bacterial Isolates (CLRBSI cases)

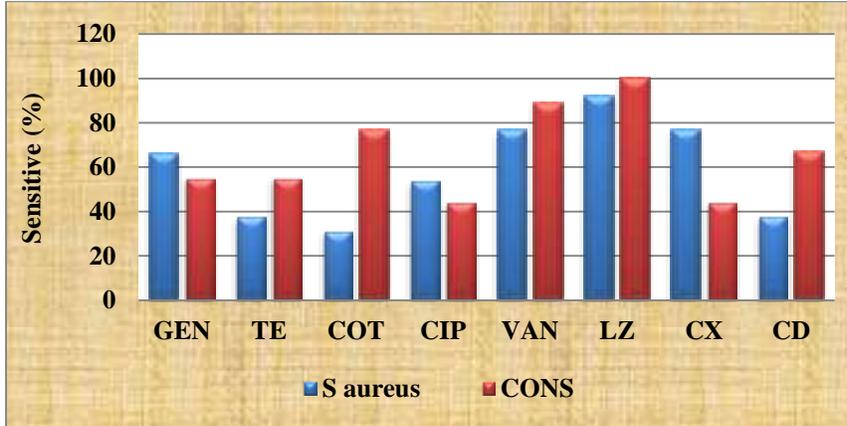
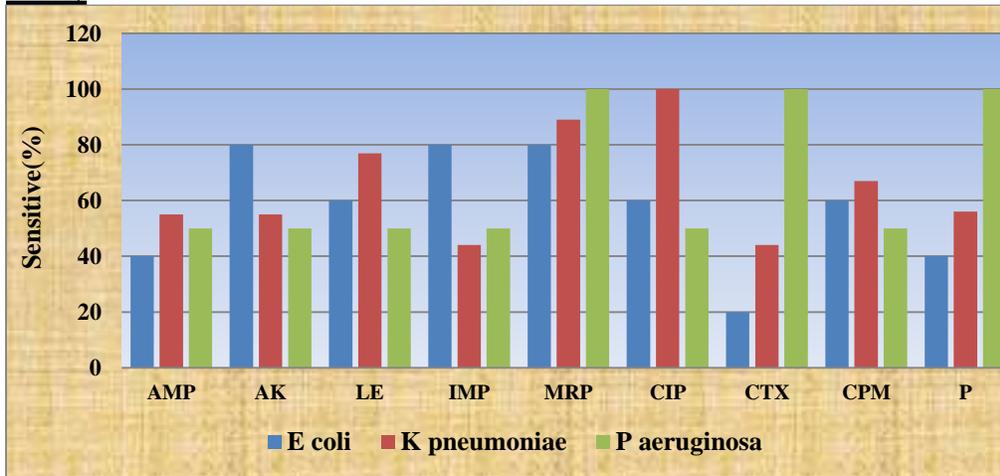


Figure:3 : Antibiotic Sensitivity Pattern of Gram Negative Bacterial Isolates (CLRBSI cases)



DISCUSSION AND CONCLUSION

In the current study out of the total 32 bacterial isolates, 10 were found to be strong biofilm producers by the CRA method of which 13 were identified as *S.aureus*. This again points to the need to follow strict hand hygiene and infection control practices.

Central venous catheters (CVCs), which include a variety of vascular access devices with a wide range of clinical applications, are disposed to biofilm infection because they offer a favourable surface for bacterial attachment [1].

Staphylococci are recognised as the most frequent bacteria responsible for biofilm-associated infections, and the involvement in catheter-related infections of meticillin-

resistant *Staphylococcus aureus* (MRSA) has increased the challenge for the healthcare community.

In present study Incidence rate per 1000 in CLRBSI was 17.5/1000 catheter days of which was almost similar to the study done by Rawal A S *et al.* [12] i.e. 18.1 but in contrast to study done by Kaur M *et al.* [13] which was 8.26 which was less than present study. This variability of incidence rate per 1000 days could be due to various factors like depends on longer duration of catheterization and critically ill patients with pre-existing systemic disease like sepsis, pneumonia, PUO. In our study most often site of catheterisation was internal jugular vein site (63%) followed by femoral vein (30%) and subclavian vein (7.0%). In contrast to present study, Harsha V patil *et al.* [14] from Maharashtra conducted a study on 137 patients with CVCs and they used subclavian vein (38.89%) and Femoral vein route (38.89%) commonly and less internal jugular vein (22.22%). These differences were attributed mainly to differential preference of central venous catheterization sites which varies across different institute due to patient's indications, ICU protocols & insertion techniques. [15] Gram positive organisms was the most common pathogen implicated in CLRBSI, which suggests colonisation of the hub either by the patient's cutaneous flora or health care personnel and reflects a probable lapse in catheter care. On anti-microbial susceptibility testing; multidrug resistance was observed in almost all the isolates responsible for CLRBSI and catheter colonisation in the present study. Biofilm formation was studied as this is inherently associated with device associated infections and contributes to antimicrobial resistance. In conclusion, CLRBSI remains an important complication of central venous access in ICUs. Active intervention of the intensivist is required to ascertain the signs of sepsis in the patient at the earliest and to send properly collected samples at appropriate time for an early diagnosis, in turn decreasing the morbidity and mortality associated with CLRBSIs.

Nevertheless, biofilm-forming capability of microorganisms is an important factor leading to the colonization of intravascular devices and dissemination of infection [16]. Central venous catheters are indispensable for the long-term treatment of seriously and chronically ill patients, but their use is often associated with a variety of complications; indeed, 90% of primary bloodstream infections are related to patients having a catheter [17,18].

The correct evidence-based nursing methods, based on the use of guidelines, provides the opportunity to minimize the risks of infection through the implementation of a series of preventive measures both during the CVC positioning phase and in the subsequent phase, for example, during device management which is performed by medical and nursing staff [19,20]. Hence, efforts should be made to raise awareness in health care workers about infection prevention practices.

DECLARATIONS:

Conflicts of interest: There is not any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors contributions: Author equally contributed the work.

REFERENCES

- 1) Mermel LA. what is the predominant source of intravascular catheter infection. Clin Infect Dis. 2011; 52(2):211-212.
- 2) Eggimann P, Pittet D. Overview of catheter related infections with special emphasis on prevention based on educational programs. ClinMicrobiol Infect 2002;8:295-309.
- 3) Donlan RM. Biofilms and device associated infections. Emerg Infect Dis 2001;7:277-81.
- 4) Maki DG, Wise CE, Safarin HW.A Semi quantitative culture method for identify IV catheter related infection. New Eng J Med. 1977; 296:1305-1309.
- 5) Eisenberg HD. Culture of intravascular devices Clinical Microbiology Procedures, Handbook 2nd Edition. Washington DC; ASM press. 2004; 13(12):1-6.
- 6) Maki DG, Wise CE, Safarin HW.A Semi quantitative culture method for identify IV catheter related infection. New Eng J Med. 1977; 296:1305-1309.
- 7) Eisenberg HD. Culture of intravascular devices Clinical Microbiology Procedures, Handbook 2nd Edition. Washington DC; ASM press. 2004; 13(12):1-6.
- 8) Cook JH, Pezzlo M. Specimen receipt and accessing. Section 1.Aerobic bacteriology, In HD Isenberg (ed)) Clinical Microbiology Procedure. Handbook. American Society for Microbiology, Washington DC. 1992; 1(2):1-4.
- 9) CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 33th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; Mar 2023.
- 10)Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol.1989; 42:872-4.
- 11)Nosocomial infection rates for inter-hospital comparison: limitations and possible solution. A report from National Nosocomial Infection Surveillance (NNIS) System. Infect Control HospEpidemiol. 1991; 12(10):609-621.
- 12) Amit Singh Rawal, Dr. Braham Prakash Sharma and Dr.Anjali Gupta. Isolation of aerobic bacteria in central venous catheter associated bloodstream infection in intensive care units of tertiary care hospital (P.B.M and A & G of Hospitals, Bikaner) & determination of antimicrobial susceptibility pattern of isolates. International Journal of Applied Research. 2018; 4(4): 308-312.
- 13) Kaur M, Gupta V, Gombar S, Chander J, Sahoo T. Incidence, risk factors, microbiology of venous catheter associated bloodstream infections A prospective study from a tertiary care hospital. Ind. J. Med. Microbiol. 2015; 33(2): 248-254.
- 14)Harsha V. Patil, Virendra C. Patil M. N. Ramteerthkar and R. D. Kulkarni. Central venous catheter-related bloodstream infections in the intensive care unit. Indian J Crit Care Med 2011; 15(4): 213-23.
- 15)Mermel LA. what is the predominant source of intravascular catheter infection. Clin Infect Dis. 2011; 52(2):211-212.
- 16) Nicola Ielapi et al. The Role of Biofilm in Central Venous Catheter Related Bloodstream Infections: Evidence-based Nursing and Review of the Literature. Rev Recent Clin Trials. 2020; 15(1):22-27.

- 17) Yazan Haddadin et al. Central Line–Associated Blood Stream Infections.2022.
- 18) B. Page *et al.* Surveillance for healthcare-associated infections: hospital-onset adult sepsis events versus current reportable conditions. *Clin Infect Dis.* 2021.
- 19) . Shallu D. National Antimicrobial Resistance Surveillance Network (NARS-Net India), Annual Report 2019; 43. Available from: ncdc.gov.in/WriteReadData/1892s/87909365291642417515.pdf. [Last accessed on 2020 Jul 17].
- 20) Arunan, Bharathi et al. Central Line-Associated Bloodstream Infections: Effect of Patient and Pathogen Factors on Outcome. *Journal of Global Infectious Diseases* . 2023; 15(2):p 59-65.