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Effect of Biofilm Formation on Antibiotic Resistance in Gram Positive Organisms Isolated from Central Line Associated and Central Line Related Blood Stream Infection at a Tertiary Care Hospital

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A B S T R A C T

Bloodstream infections (BSIs) are the most common type of HAI occurring in the ICU. Infections associated with CVCs are categorized in the literature as either "Central line related bloodstream infections (CLRBSI), or central line associated blood stream infection (CLABSI) based on whether surveillance or ascertainment of infection is the desired goal. Blood stream infection related to central venous Catheter constitutes one of the major nosocomial device associated infection. To isolate the organisms that causing CLABSI & CLRBSI. To Determine Antimicrobial Susceptibility of the isolates & its relevance to biofilm formation. Catheter tips were processed using Extraluminal Maki's roll over method and Endoluminal catheter flush culture methods. And simultaneously obtaining blood culture from peripheral site & from the CVC. Biofilm formation was detected by using tissue culture plate (TCP). Blood Samples and Catheter tips were collected during the period 3 year from September 2021 to September 2024 at SSB Hospital & NMC Hospital, Government Medical College, Kota, Rajasthan. A total of 155 patients with a cumulative 1380 CVC days were included. Antibiotics resistance was seen more in Biofilm producing bacteria as compared with Non biofilm producing bacteria. Catheter colonization and duration of catheterization has an important role in development of CLRBSI and CLABSI which may lead to septicaemia and multiorgan failure. The findings from this study will help to implement educational programs on CLRBSI/CLABSI for health personnel and help to initiate infection control interventions at the earliest.

Introduction

Central venous catheters (CVCs) have become essential in the management of critically ill patients, as well as other patient populations who require long-term medical care. Central venous catheters are used to access the vascular system for the delivery of medication, parenteral nutrition, the collection of blood samples and haemodynamic monitoring (Klevens *et al.,* 2002). Bacterial colonization of the device and the contamination of infusate are primary causes of CLABSI. Colonization of the device may be either extra luminal (from surrounding skin or haematogenous seeding of the catheter tip) or intraluminal (due to biofilm formation by an organism leading to persistence of infection and haematogenous spread). Although rarer, it is contaminated infusate that leads to the majority of epidemic intravascular device-related BSIs (Rao *et al.,* 2011). Central venous catheter-related blood stream infections (CRBSI) are of particular interest as

indwelling vascular catheters have been shown to be responsible for about 62% of ICU acquired blood stream infections which added to the morbidity and mortality of ICU stay (Maki *et al.,* 1977). In addition, CRBSI has been shown to increase both ICU and hospital length of stay (Heard *et al.,* 1998). CVCs have a higher infection risk than other indwelling vascular access lines. This causes significant morbidity and mortality to the critically ill patient (Dimick *et al.,* 2001).

The main aim and objectives of this study includes to identify bacteria that causing CLABSI & CLRBSI. To determine the rate of CLABSI & CLRBSI per1000 catheter days. And also, to Determine Antimicrobial Susceptibility of the isolates & its relevance to biofilm formation.

Materials and Methods

This Prospective study was carried out at department of Microbiology, Government medical college kota, Rajasthan, over a period of 3 years (September 2021 – September 2023). The study was approved by the ethical committee of Institute. All Patients with age >18 years admitted in the Medical ICU, with central line for more than 48 hours (2 calendar days) having signs & symptoms of infection fever, chills and hypotension were included. Patients with CVC, having obvious other source of infection were excluded.

Collection of blood sample and processing

Blood sample collected under aseptic precautions from the peripheral vein as well as central venous catheter & inoculated onto BHI Biphasic Blood culture media for qualitative culture and incubated aerobically at 37º C for 1 to 7 days. The bottles were examined for turbidity daily. Subculture was done onto MAC, BA, and Sabouraud Dextrose Agar slant & incubated aerobically at 37º C for 24 to 48 hours (Cook and Pezzlo, 1992).

The CLABSI/CLRBSI rate was calculated by the following formula: (Book of scientific proceedings of national cme cum workshop hospital infection prevention & control practices and antimicrobial stewardship program, 2017)

$$\text{CLABSI/CLRBSI rate per 1000 catheter days} = \frac{\text{No. Of CLABSI /CLRBSI cases x 1000}}{\text{No. of CVC days}}$$

Collection of Central line tip and processing

Catheter tip were processed by using both Semiquantitative Extraluminal Maki's roll over method & Quantitative Endoluminal catheter flush culture methods. In Maki's roll over method catheter tip was transferred from transport container to agar plate using sterile forceps. The catheter tip was rolled back and forth across agar surface using slight pressure at least four times. It was made sure that the catheter tip was having good contact with the surface of the plate. The plates were incubated aerobically at 37ºC for 48- 72hr. & colonies counted. Significant growth is defined as ≥ 15 colony forming units (CFU) (Deshpande *et al.,* 2005). In Endoluminal catheter flush culture method One mL of sterile normal saline was flushed in to the lumen of the segment using a sterile syringe and 0.01 ml of the suspension inoculated each onto BA, MAC and SDA slant respectively. The plates were incubated aerobically at 37ºC for 24 to 48 hours. Significant growth is defined as ≥ 10^3 CFU/ml (McGee and Gould, 2003).

Identification of Microorganisms

The Gram positive organisms were identified by Colony characteristics, Pigment production Gram staining, Morphology & arrangement of cocci, and Biochemical tests such as Catalase, & Coagulase (Both Tube & Slide) test (Chopdekar *et al.,* 2011).

Antimicrobial susceptibility testing

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) (CLSI, 2023).

Biofilm detection by Tissue culture plate method (TCP)

Isolates from fresh agar plates were inoculated in respective media (BHI Broth with 2% sucrose) and incubated for (18- 24) hours at 37˚C. The culture was further diluted 1:100 with fresh medium 96 wells flat bottom tissue culture plates were filled with 0.2 ml of diluted cultures individually. Only sterile broth was served as blank. The tissue culture plates were incubated for 18-24 hours at 37˚C after incubation content of each

well was gently removed by tapping the plates. The wells were washed four times with 0.2ml of phosphate buffer saline (pH 7.2) to remove free-floating plankiton bacteria. Biofilm formed by adherent organisms in plate were fixed with sodium acetate (2%) and stained with crystal violet (0.1 %.) Excess stain was rinsed off by washing with deionized water and wells OD were determined with a micro ELISA auto reader at wave length 490 nm (Stepanovic *et al.,* 2007).

1. Mean OD = <0.120 (Non/Weak Biofilm production).
2. Mean OD = 0.120-0.240 (Moderate Biofilm production).
3. Mean OD = >0.240 (Strong Biofilm production)

Results and Discussion

In present study total 155 cases studied out of which 92 (59.35%) were male and 63 (40.64%) were female. So male to female ratio was 1.46:1. In present study the mean age of the patients was 60.4 years; male patients were more as compared to female. Most of the patients belonged to the age group of 61-70 years were 56(36.12%) of which 31(55%) and 25(44%) were males and females respectively. In present study out of 52 bacterial isolates among (CLABI/CLRBSI/Tip colonization), *S aureus* were 27(52%) and 25(48%) were CONS. All 52 isolates were tested for biofilm production by Tissue culture plate method. The results were categorize biofilm formation into three groups: Strong (S), Moderate (M), and Weak/none (W/N). Among Staphylococcu *S aureus* (n=27) isolates, 9 (33%) demonstrated strong biofilm formation, while 5 (18%) showed moderate biofilm formation, and 13 (48%) exhibited weak or no biofilm formation. Coagulase-Negative Staphylococcus (CONS) (n=25), exhibited higher percentage (40%) of isolates showed moderate biofilm formation, with 36% strong and 24 % considered as weak or non-biofilm producers..

In present study Incidence rate per 1000 in CLABSI was 16.12 /1000 catheter days of which was almost similar to the study done by Rawal A S *et al.,* from Bikaner i.e. 19.1 and Kaur M *et al.,* from Chandigarh i.e 14.59 but in contrast to study done by Abirami E *et al.,* from Chennai i.e. 8.26 which is less than present study. The lower rates could be attributed to a poor nurse-to-patient ratio, compromised infection control practices, and the critically ill patients with pre-existing sepsis in the CVC-BSI group. In present study TCP method showed 63% biofilm production which was almost similar to the study done by Mathur T *et al.,* from New Delhi, Krithiga R from Tamil nadu i.e. 54% and 63% respectively.

But in contrast to study done by Malvika S *et al.,* from Jaipur i.e. 82% which was higher than present study. Malvika stated that the differences in the sources from which the strains were isolated and differences in the methodology employed in the study and TCP methods provide a larger surface area for biofilm growth. In present study Biofilm producer's *S aureus* (BPSA) showed the highest resistance to Clindamycin (79%), Gentamicin (79%), Cotrimoxazole (72%), and Erythromycin (72%). In contrast, non-biofilm producing *S aureus* demonstrated notably lower resistance levels for these antibiotics, with resistance rates of 31% for Clindamycin, 15% for Gentamicin & Cotrimoxazole and 23% for Erythromycin. Biofilm producer's CONS (BPCONS) notably higher resistance to Cefoxitin (58%), Gentamicin (58%), Clindamycin (47%), and Cotrimoxazole (42%). In contrast, non-biofilm producers showed significantly lower or no resistance to these antibiotics, with 0% resistance observed for Gentamicin, Clindamycin, Cotrimoxazole and 16% for Cefoxitin.

Biofilm forming bacteria generally show a greater resistance to antibiotics than Non biofilm forming bacteria because of the difficulty in penetration of drugs through the biofilm. Many studies have been undertaken which reported high resistance among different biofilm forming bacteria. Most of the study results were similar to the present study but some differences in sensitivity to antibiotics were seen. Different authors have performed studies on different clinical samples and antibiotic susceptibility pattern vary with the geographical area and hospital environment.

In the present study duration of central venous catheter more than seven days & was associated with higher catheter colonization rate. Similar findings were observed by in a study by Sato *et al.,* (2017) where the incidence of infection increased within 8-10 days of catheterization (Sato *et al.,* 2017). Catheter tip colonization may result from health care interventions and constitutes an important cause of morbidity and mortality among ICU patients Simple preventive measures, such as aseptic precaution during catheter insertion, daily catheter care, monitoring of catheterised patients, could help to reduce risk of colonisation and subsequent catheter related infections.

Table.1 Blood culture and CVC tip culture results

Blood culture	Tip culture	Impression	No of cases	Rate per 1000 central line days
Positive	Positive	CLRBSI	9 (5.8%)	6.5
Positive	Negative	CLABSI	25 (16.12%)	18.11
Negative	Positive	Tip colonization	18 (11.61%)	-

Table.2 Microorganism profile of CLRBSI, CLABSI & Tip colonization

Isolates	CLRBSI	CLABSI	Tip colonization	Total %
S aureus	05	15	07	27(52%)
CONS	04	10	11	25(48%)

Table.3 Detection of Biofilm productions by Tissue culture plate (TCP) method

Organisms tested for biofilm	Tissue culture plate method		
	S	**M**	**W/N**
S aureus **(n=27)**	9 33%	5 18%	13 48%
CONS (n=25)	9 36%	10 40%	6 24%

Table.4 Correlation between Antibiotic resistance pattern with Biofilm producer (BP) and Non Biofilm producer (NBP) of Gram positive cocci

Resistant Isolates (%)	***S aureus*** **(27)**		**CONS (25)**	
	BP (14)	**NBP (13)**	**BP (19)**	**NBP (06)**
AMP	3(22)	1(8)	3(19)	0(00)
AMC	6(43)	2(15)	6(32)	1(16)
COT	10(72)	2(15)	8(42)	0(00)
CD	11(79)	4(31)	9(47)	0(00)
CX	8(57)	1(8)	11(58)	1(16)
E	10(72)	3(23)	9(47)	5(83)
LZ	1(7)	2(15)	3(19)	1(16)
VA	1(7)	1(8)	1(5)	1(16)
LE	5(36)	3(23)	9(47)	4(66)
GEN	11 (79)	2(15)	11(58)	0(00)

Figure.1

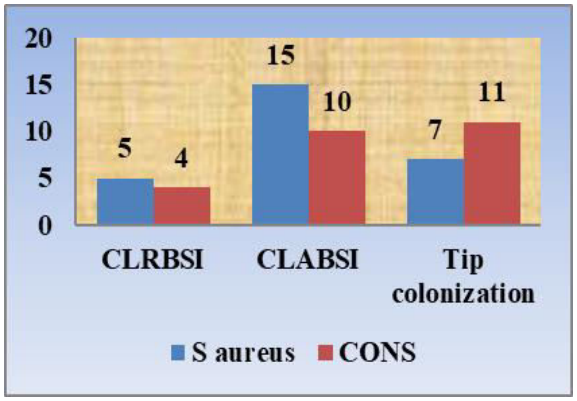


Figure.2

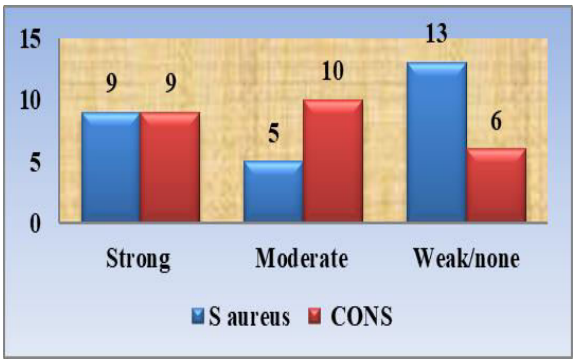


Figure.3 Correlation between Antibiotic resistance pattern with Biofilm producer (BP) and Non Biofilm producer (NBP) of *S aureus* isolates

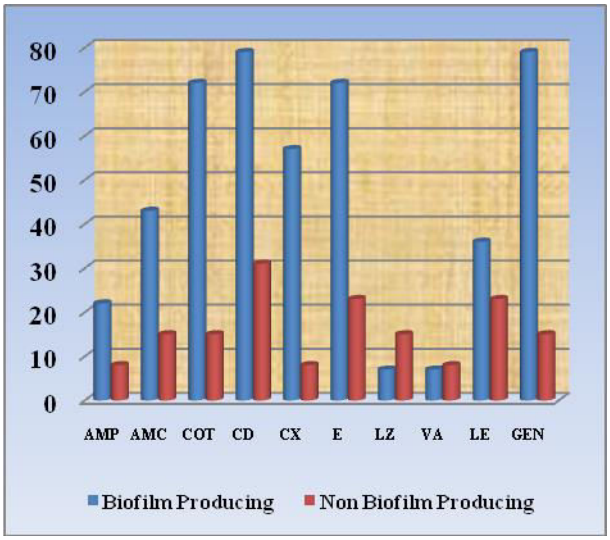


Figure.4 Correlation between Antibiotic resistance pattern with Biofilm producer (BP) and Non Biofilm producer (NBP) of CONS isolates

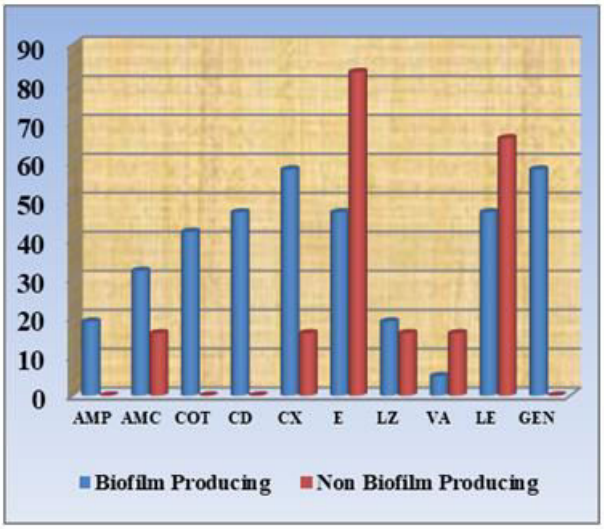
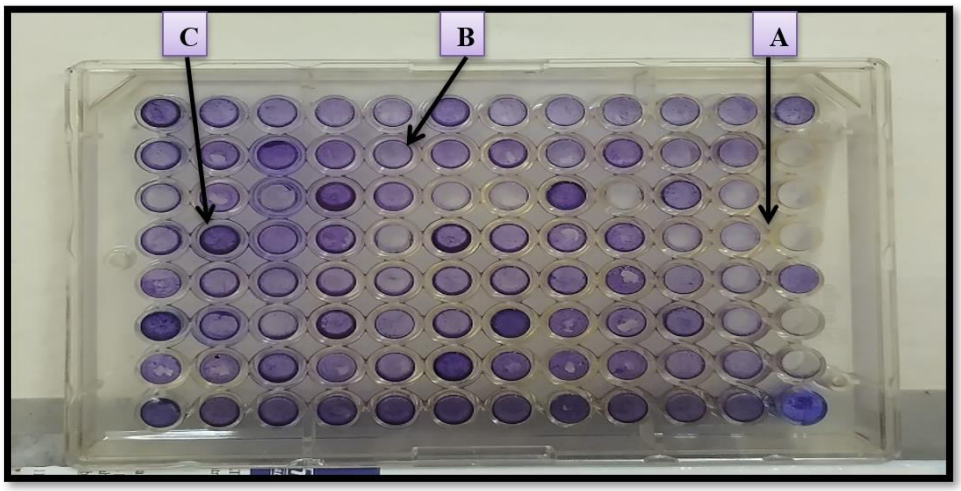


Figure.5 Detection of biofilm production by tissue culture plate method (TCP).



A: Non /week biofilm producer,
B: Moderate producer biofilm,
C: Strong producer biofilm producer.

Prevention of CLABSI/CLRBSI is essential to improve the outcomes and prognoses of MICU patients. It is most important to regularly educate, train and give feedback to medical staffs about the various preventive measures required at each stage from before insertion to removal of the catheter. Effective management requires biofilm-targeted therapies, combination antibiotics, and preventive strategies to reduce biofilm-associated complications. Routine screening for biofilm formation and antimicrobial stewardship are crucial for improving treatment outcomes.

Author Contributions

Dharmendra Singh: Investigation, formal analysis, writing—original draft. Anita E. Chand: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval: Approval from Institutional Ethical Committee (IEC) was obtained prior to commencement of the study from Government Medical College Kota (Raj.)..

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.

Source of funding: None

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