Original Article

Central Line-Associated Bloodstream Infections: Effect of Patient and Pathogen Factors on Outcome

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Abstract

Introduction: Patients on central lines are often having multiple morbidities, and invasive devices provide a niche for biofilm formation, which makes central line-associated bloodstream infections (CLABSIs), a serious concern in health-care settings, as the infections difficult to treat. In this study, we evaluated the common bacteria causing CLABSI, and various patient and pathogen factors affecting the clinical outcome. **Methods:** In the prospective observational study, patients diagnosed with CLABSI were recruited. Extensive clinical, microbiological, and other laboratory workup was done, and observations were recorded. Congo red agar method, tube test, and microtiter plate assay were used for eliciting the biofilm-forming attributes of the bacterial pathogens. **Results:** *Klebsiella pneumoniae* was responsible for 48% of CLABSI, followed by Coagulase-negative *Staphylococci* (16%) and *Staphylococcus aureus* and *Acinetobacter baumannii* (12% each). Fifty-six percent of the isolates produced biofilms. The median (interquartile range) duration of hospital stay till death or discharge was 30 (20, 43) days. The all-cause mortality was 44%. Patients having a deranged liver function on the day of diagnosis (*P* value for total bilirubin 0.001 and for aspartate transaminase 0.02), and those infected with multidrug-resistant organisms (*P* value = 0.04) had significantly poor prognosis. The difference in the demographic, clinical, laboratory profile, and outcome of patients infected with biofilm producers and nonproducers was not found to be statistically significant. **Conclusion:** The study throws light on various host and pathogen factors determining the cause and outcome of CLABSI patients. To the best of our knowledge, this is the first study trying to decipher the role of biofilm formation in the virulence of pathogens and the prognosis of CLABSI.

Keywords: Antimicrobial resistance, biofilm formation, central line-associated bloodstream infections, central line

Introduction

Bloodstream infections are a serious concern in health-care settings, especially with the increase in the use of invasive devices in critically ill patients. The current incidence in India, according to the International Nosocomial Infection Control Consortium, has been reported to be around 4.11 per 1000 central line days, while regional studies report an incidence ranging from 2.3 to 13.8 per 1000 central line days.[1,2] Due to the morbidity, these infections pose. The Center for Disease Control (CDC) has introduced a surveillance definition, aiding in their early diagnosis and management.[3]

Biofilms are colonies of microorganisms surrounded by an exopolysaccharide matrix. Almost 80% of infections caused in humans such as dental caries, otitis media, chronic sinusitis, endocarditis, and urinary tract infections are associated with biofilm formation.[4,5] Invasive devices form a niche for biofilm production. The device-related infections can range from prosthetic joint infections to the central line-associated bloodstream infections (CLABSI).[6] A multitude of factors such as delayed penetration of antimicrobials into the biofilm, altered growth rate of bacteria, and immune evasion by the microorganisms make them difficult to treat.[7]

The link between biofilms and infections has been established, but their implication on the clinical course of the infection has not been well defined. In posttraumatic infected wounds, biofilm production has been shown to be an important virulence

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/jgid.jgid_213_22

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How to cite this article: Arunan B, Ahmed NH, Kapil A, Vikram NK, Sinha S, Biswas A, *et al.* Central line-associated bloodstream infections: Effect of patient and pathogen factors on outcome. J Global Infect Dis 2023;15:59-65.

Received: 09 November 2022 **Revised:** 03 January 2023
Accepted: 02 February 2023 **Published:** 31 May 2023

factor, leading to delayed healing.[8,9] Strains of *Escherichia coli* causing chronic urinary tract infections usually have potent biofilm-forming capability.[10] Such studies for other device-related infections, such as CLABSI are lacking.

The use of central lines for indications such as hemodialysis, ionotropic support, administering chemotherapeutic drugs, or total parenteral nutrition is common practice in our tertiary care center, making CLABSI a common occurrence. Factors which influence the outcome of CLABSI include comorbidities such as diabetes mellitus, chronic kidney disease, immunosuppressed states, and infection by virulent multidrug-resistant (MDR) organisms.[11] We aimed to study the common bacteria causing CLABSI, their antimicrobial profile, the biofilm-forming ability, and the patient factors affecting the clinical outcome of these infections. We also compared three tests for studying the biofilm-forming attributes of the bacterial isolates.

Methods

Study design and setting

This was a prospective observational study conducted in a tertiary healthcare institute in New Delhi, India, between July 2019 and May 2021. This study was approved by the Institutional Ethics Committee (IECPG-498/July 17, 2019). The authors followed applicable EQUATOR Network guidelines, i.e. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines during the conduct of this research project.

Participants

The patients were admitted to wards and intensive care unit (ICU) under the department of medicine. Considering the prevalence of laboratory-confirmed CLABSI ranging from 22 to 43 over the past years, a sample size of 25 was arrived at for the study. Written informed consent was obtained from all patients or caregivers. The criteria for the inclusion in this study were patients aged more than 18 years and diagnosed with CLABSI according to the CDC surveillance definition.[3] The patients who had other identifiable sources of infection, like respiratory tract infections, urinary tract infections, infective endocarditis, meningitis, etc., and those <18 years of age were excluded from the study.

Detailed history and thorough physical examination were done by infectious disease experts. Relevant laboratory parameters were monitored, and follow-up was done until death or discharge. Clinical and laboratory data were collected, both by direct observation and through electronic health records. The data were entered into a pro forma approved by the IEC.

Microbiological processing

Ten milliliters of blood, each from the central line and peripheral vein were drawn under sterile precautions and inoculated into adult BD BACTEC™ Plus Aerobic culture bottles. Samples from the flagged bottles were subcultured onto MacConkey agar and blood agar for colony growth and identification. Further bacterial speciation was done using matrix-associated laser desorption ionization-time of flight, and antibiotic susceptibility testing (AST) was done by the Kirby–Bauer disk diffusion method.

Tests for biofilm production

All bacterial isolates were stored in a nutrient butt at −20°C and revived for tests of biofilm production. A positive control (*Staphylococcus epidermidis* ATCC 35984), a negative control (*S. epidermidis* ATCC 12228), and a sterile control were used with each of the tests. Biofilm formation attributes of the bacterial isolates were tested using the three following methods:

(a) The Congo red agar (CGA) method was done by plating a loopful of bacteria, in log phase, on CGA. The plates were read after incubation at 37°C for 24 h. A biofilm producer was identified by black crystalline colonies, whereas nonproducers grew as translucent colonies [Figure 1a and b].[12]

(b) The tube test was carried out in test tubes containing the bacterial isolate inoculated in trypticase soy broth with 1% glucose. The test tubes were incubated at 37°C for 24 h. Then, they were decanted and washed with phosphate buffer saline (PBS). The biofilms formed at the bottom of the tube were identified by staining with 0.1% crystal violet. Depending on the intensity of the stain, the isolates were classified as nonbiofilm producers (0),

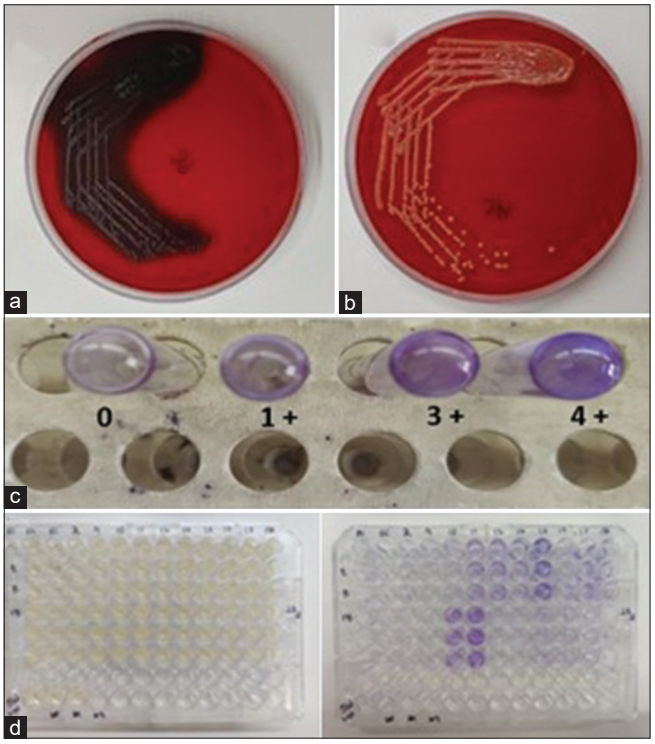

Figure 1: Methods of testing biofilm formation attributes of the pathogens: (a) Congo red agar showing black colonies with dry crystalline consistency indicating a biofilm-producing bacteria; (b) Congo red agar showing colonies of non-biofilm producing bacteria; (c) Test tube method for biofilm formation showing the range from nonbiofilm producer (0) to strong biofilm producer (4+); (d) microtiter plate assay

weak (1+), moderate (2+), and strong (3 + and 4+) biofilm producers [Figure 1c].[12]

(c) The third test was the quantitative microtiter plate assay, the gold standard in our study. The bacterial isolate was inoculated on a presterilized polystyrene 96-well microtiter plate with the culture media (trypticase soy broth with 1% glucose). After incubation, washing with PBS and staining with 1% crystal violet was done, and the plates were read using an ELISA reader at 570 nm. The optical density (OD) readings were used to classify bacteria as non, weak, moderate, and strong biofilm producers [Figure 1d].[12] The following calculations were used to calculate the OD cutoffs.[13]

OD (control) = average OD of negative control + (3 × standard deviation (SD) of negative control)

Nonproducer = OD ≤ ODc

Weak producer = > ODc ≤2 × ODc

Moderate producer = >2 × ODc ≤4 × ODc

Strong producer = >4 × ODc

Statistical analysis

The data were entered and maintained in a Microsoft Excel (2019) Sheet, and GraphPad Prism Version 8.4.2 was used for the statistical analysis. Continuous variables were represented using median and interquartile range (IQR) or mean and SD. Categorical variables were represented as number (percentages). MannWhitney *U* and unpaired *t*-test were used to compare continuous variables and Chi-square test for categorical variables. A $P = < 0.05$ was considered statistically significant.

Results

Demographic profile and clinical profile of the participants

A total of 25 patients were included in the study. The mean (SD) age of the population was 40.5 (16.8) years and 56% were males. Forty-four percent of the patients suffered from chronic kidney disease and 40% from diabetes mellitus [Table 1]. The triple lumen central venous catheter was the most common type of central line used (68%), with the right internal jugular vein being the most common site of insertion (48%).

The most common clinical presentation indicating a bloodstream infection was fever, seen in 80% of the patients. Local site inflammation was present in 44%, and 36% of the individuals had new onset hypotension. On routine investigations, neutrophilic leukocytosis was the most common laboratory finding observed, occurring in 68% of the subjects. The median (IQR) leukocyte count on the day of the event was 10,900 (8700–17,600) and creatinine was 2.3 (0.6–4.7) [Table 2].

Bacterial isolates

The most common pathogenic bacteria were *Klebsiella pneumoniae*, responsible for 12 cases (48%) of CLABSI.

Table 1: Demographic profile of the study participants

Parameter	CLABSI patients (*n*=25), *n* (%)
Age (years), mean (SD)	40.5 (16.8)
Sex	
Male	14 (56)
Female	11 (44)
Comorbidities	
Diabetes mellitus	10 (40)
Hypertension	8 (32)
Chronic kidney disease	11 (44)
Chronic liver disease	3 (12)
Immunocompromised state	2 (8)
Others	2 (8)

CLABSI: Central line-associated bloodstream infection, SD: Standard deviation

Table 2: Laboratory parameters of the study participants

Parameter	Value
Total leukocyte counts on DOE	
Mean (SD)	13,708 (8219.1)
Median (p25-p75)	10,900 (8700-17,600)
Creatinine on DOE	
Mean (SD)	3.1 (2.7)
Median (p25-p75)	2.3 (0.6-4.7)
Hemoglobin on DOE	
Mean (SD)	7.9 (1.7)
Median (p25-p75)	7.9 (6.8-8.8)
Platelet on DOE	
Mean (SD)	1,58,960 (1,32,078)
Median (p25-p75)	1,20,000 (70,000-1,77,000)
INR on DOE	
Mean (SD)	1.4 (0.3)
Median (p25-p75)	1.32 (1.2-1.5)
Total bilirubin	
Mean (SD)	1.24 (2)
Median (p25-p75)	0.4 (0.3-1.1)
AST	
Mean (SD)	61.9 (103.6)
Median (p25-p75)	30 (16-77)
ALT	
Mean (SD)	47.1 (70.5)
Median (p25-p75)	20 (9-57)

DOE: Day of event, SD: Standard deviation, INR: International normalized ratio, AST: Aspartate transaminase, ALT: Alanine transaminase

Coagulase-negative *Staphylococci* (CoNS) were implicated in four (16%) of the infections. *Staphylococcus aureus* and *Acinetobacter baumannii* each caused 3 (12%) of cases, whereas, *Chryesobacterium indologenes*, *E. coli,* and *Enterococcus faecium* caused one bloodstream infection each [Figure 2].

AST was done using the Kirby–Bauer disk diffusion method, and interpretation was done using clinical and laboratory standard institute (CLSI) guidelines. Gram-negative isolates

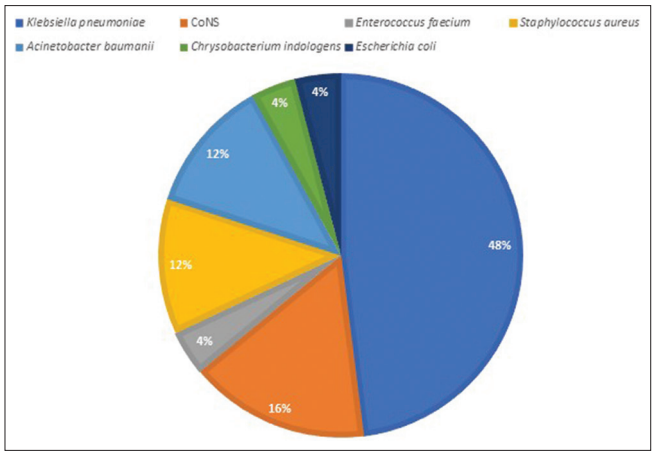

Figure 2: Bacteriological profile of CLABSI patients. CLABSI: Central line-associated bloodstream infections

were considered MDR if resistance was detected to more than three classes of antibiotics. This included extended-spectrum beta-lactamase producing and carbapenem-resistant *Enterobacterales*, difficult-to-treat *Pseudomonas* and carbapenem-resistant *Acinetobacter. Staphylococci* sp. were classified as MDR if they were cefoxitin resistant, which is a surrogate marker for methicillin resistance, rendering the isolate resistant to penicillins, cephalosporins, older beta-lactam-beta-lactamase inhibitor combinations, carbapenems, and aztreonam. Vancomycin-resistant *Enterococcus* sp. (VRE) was considered as MDR.

Resistance pattern of the bacterial isolates

Antibiotic susceptibility of the bacterial isolates was done for drugs recommended by CLSI. Nearly 94.1% (16/17) of the Gram-negative organisms were MDR, and all of them were carbapenem resistance on phenotypic testing. However, all the isolates were susceptible to colistin. The Gram-positive isolates included four methicillin-resistant and three methicillin-susceptible *Staphylococci* sp. None of the strains were vancomycin intermediate or resistant. The *E. faecium* isolate was resistant to penicillin and vancomycin [Figure 3]. We also compared the susceptibility pattern of the biofilm-producing and nonproducing isolates, and the difference was not statistically significant.

Biofilm production

For biofilm production, three tests, namely, (i) CGA method, (ii) tube test, and (iii) microtiter plate assay were carried out. The results of the microtiter plate assay were considered gold standard. Fourteen of the 25 isolates (56%) were found to be biofilm producers. The sensitivity, specificity, positive predictive (PPV), and negative predictive value (NPV) of the congo red agar (CRA) method were 66.7%, 44.4%, 54.6%, and 57.1%, respectively. The tube test had a sensitivity of 58.3%, specificity of 33.3%, PPV of 63.6%, and NPV of 28.6%, respectively.

87.5% of the Gram-positive bacterial isolates were biofilm producers in contrast to only 41.2% of the Gram-negative bacterial isolates (P = 0.04). All four CoNS and 66.7% of

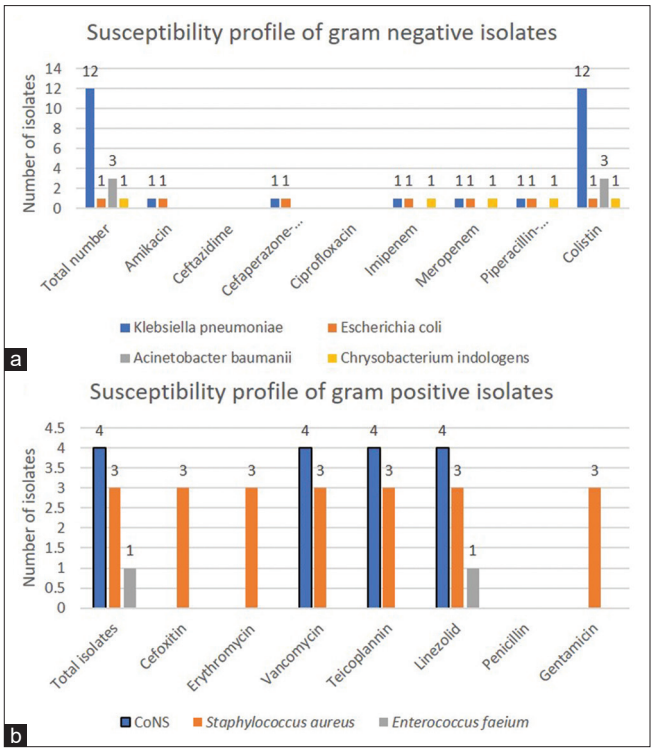

Figure 3: Susceptibility profile of the bacterial isolates causing CLABSI; (a) Gram-negative isolates; (b) Gram-positive isolates. CLABSI: Central line-associated bloodstream infections

S. aureus isolates produced biofilms. Of the Gram-negative isolates, 41.7% of *K. pneumoniae* and 66.7% of the *A. baumanii* bacterial isolates produced biofilm [Figure 4].

The demographic, clinical, laboratory profile, and outcome of the patients infected with biofilm producers and nonproducers were compared. The difference was not found to be statistically significant [Table 3].

Outcome

In the study population, the median (IQR) duration of hospital stay was 30 (20, 43) days. The all-cause mortality was 44% (11 of 25 patients). The patients who succumbed to the infection had, statistically significant, higher total bilirubin (P = 0.001), and aspartate transaminase (P = 0.02), on the day of diagnosis. In addition, infection caused by MDR organism was associated with higher mortality (P = 0.04). Of note, all five patients who were infected with non-MDR organisms survived.

Discussion

Bloodstream infections or bacteremia usually present with systemic symptoms such as fever and hypotension. The surveillance definition of CLABSI according to CDC, also states these signs as criteria for diagnosis.[3] In our study, 80% of the cases diagnosed with CLABSI, presented with fever, and 36% had hypotension. Shin *et al*. in a follow-up study showed that fever had an odds ratio (OR) of 4.78 for predicting the development of bloodstream infection.[14] Purulent exit site, according to expert opinion, should also strongly raise

Table 3: Demographic profile and comorbidities of patients with infections caused by biofilm-producing and nonbiofilm-producing bacteria

Parameter	Biofilm producers (*n*=14)	Biofilm nonproducers (*n*=11)	*P*
Age (years), median (p25-p75)	38 (21.5-56.2)	45 (23-56)	0.70
Sex (%)			
Male	42.9	45.5	0.99
Female	57.1	54.5	
Comorbidities (%)			
Diabetes mellitus	14.3	72.7	0.01
Hypertension	14.3	54.5	0.08
Chronic kidney disease	42.9	45.5	0.99
Chronic liver disease	14.3	9.1	
Immunosuppressed	7.1	9.1	
Cerebrovascular accident	0	9.1	
Coronary artery disease	0	9.1	

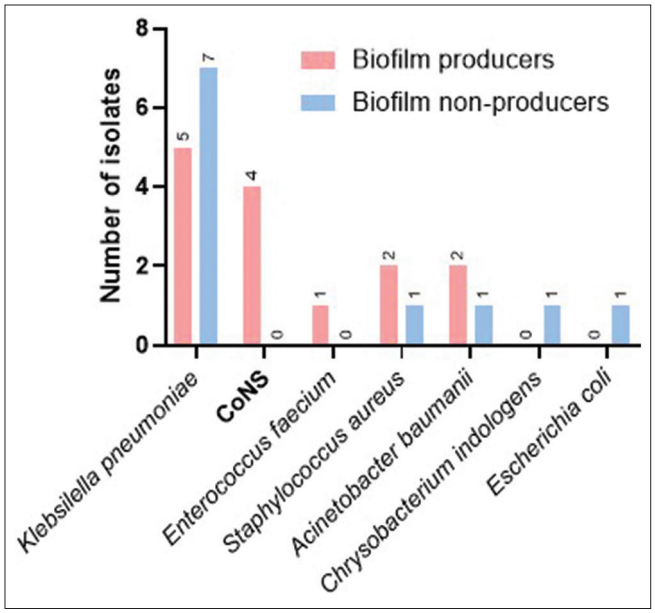

Figure 4: Biofilm production attributes of different pathogenic bacterial species

suspicion for infection. Complicated catheter infection may be predicted if hemodynamic instability, local exit signs, or neutrophilia is present.[15]

The risk factors leading to the development of CLABSI include chronic kidney disease, patients with extensive burns, and those requiring chemotherapy.[16] The incidence in chronic kidney disease patients on hemodialysis is about 17.7 episodes per 100 person-years, translating to almost a 26 times higher incidence than in the general population.[17] Other comorbidities such as type II diabetes mellitus and chronic liver disease are also frequent.[16] In our study, chronic kidney disease and diabetes mellitus were the most common. Another important factor considered in the surveillance of CLABSI is the number of central line days before the development of infection. In the present study, it ranged from 3 to 90 days, with a median of 10 days. This is akin to the published literature, with the median days ranging between 9 and 61 days.[18]

Recent data have shown Gram-negative multidrug-resistant bacteria more commonly lead to CLABSI than Gram-positive skin commensals.[19] Various studies from India have shown that hospital-acquired pathogens, namely, *K. pneumoniae, Pseudomonas aeruginosa,* and *A. baumannii* are commonly implicated.[20] This is in contrast to data from NHSN, which states a dominance of *Staphylococcus* sp. (56%) followed by Gram-negative bacteria and fungi.[21] *C. indologenes* was an uncommon causative organism in one of our study subjects. It is a nonmotile, Gram-negative rod, known to be resistant to chlorine treatment. It can colonize hospital water supplies and infusions. Six infections caused by *C. indologenes* have been described in our institute, with the type of infections ranging from the respiratory tract to bloodstream infection. Infections by this organism in patients with intravascular devices are usually associated with biofilm production.[22]

Most of our isolates were MDR, including 94.1% of the Gram-negative and 62.5% of the Gram-positive bacterial isolates. Of concern was the high prevalence of carbapenem resistance (94.1%) in the hospital-acquired Gram-negative bacteria. These rates are much higher than the ICMR surveillance data for 2020, where a resistance of about 30%–50% for *Enterobacterales* and 50%–70% for nonfermenting Gram-negative bacteria has been documented.[23] None of the *Staphylococcus* sp. was vancomycin resistant, which is similar to that reported by other Indian studies. The single *Enterococcus* isolate in our study was a VRE. Between January and December 2020, 9% of *Enterococcus* isolates from India were found to be vancomycin resistant.[23]

On studying the biofilm formation attributes, Gram-positive bacteria were found to be more commonly associated with the formation of biofilms than Gram-negative bacteria. This finding is in concordance with existing literature where the biofilm formation ability of Gram-positive isolates is well documented.[24,25] A multivariate analysis by Barsoumian *et al*. has shown that infections caused by *E. coli*, *P. aeruginosa,* or MR-*S. aureus* were independent risk factors for biofilm production.[9] Multiple studies evaluating chronic wounds, a niche for biofilm production, found biofilm-forming *S. aureus* to be a major risk factor for their persistence.[26] Similarly, an *in situ* device also provides a niche for the formation of biofilms. This provides an additional advantage to biofilm-producing strains and leads to persistent, difficult-to-treat infections. This was demonstrated by Babushkina *et al*., who found that clinical strains of *Enterobacterales* causing implant infections had more prominent biofilm production than those aspirated from pus.[6]

When compared with the microtiter assay, the CRA method showed moderate sensitivity and poor specificity. Previous studies on biofilm production have reported varied results.

Studies on more than 100 strains of *Staphylococcus* sp., reported a very poor sensitivity <10% with a higher specificity of around 90%.[24] Arciola *et al*. compared the CRA method with polymerase chain reaction (PCR) for *icaA* locus of *Staphylococcus* sp., which is responsible for the biofilm-producing attribute. The results of both the tests were found to be comparable; hence, they concluded that CRA could be a rule in test.[25] The poor specificity in our study maybe due to the fact that the utility of CRA method with Gram-negative isolates is yet to be ascertained. Of the three *S. aureus* isolates we tested, two had similar results in CRA and microtiter plate assay. The sensitivity and specificity for the tube test as compared to microtiter plate assay is reported to be 76% and 97%, respectively.[24] Weak producers as reported in microtiter method can be missed by the tube test. *S. aureus* isolates had a good correlation of tube test and tissue microtiter plate assay.

In the present study, we found no significant difference in the antimicrobial resistance between the biofilm producers and nonproducers. Prominent literature states that one of the most worrisome features of biofilm infections is its antimicrobial resistance. The mechanisms for this resistance are postulated to be multifaceted including delayed penetration of antimicrobial, altered growth rate of microorganism, and increased expression of resistance genes in the environment.[7] The reason for our biofilm-producing and nonproducing isolates showing similar susceptibility pattern may be explained by the method of AST. In this study, we used the Kirby–Bauer disk diffusion method, where the inoculum is in the planktonic stage. Broth microdilution assays where the bacteria are in the log phase and have an interface for biofilm formation maybe better suited.[27]

The all-cause mortality from CLABSI was 44%; this is similar to the published rates of death from hospital-acquired bloodstream infections, which range from 45% to 70%.[20,28] An analysis of 166 cases by Atilla *et al*. reported that infection with *Candida* sp. and a higher APACHE II score on admission were independent risk factors for mortality.[16] In our analysis, underlying deranged liver function tests and infection due to an MDR organism showed a predilection for mortality. As the values of liver parameters are of the day of diagnosis of CLABSI, the out-of-range liver function tests can also be a consequence of multiorgan dysfunction occurring due to sepsis, which is independently known to add to poor prognosis. Furthemore, the duration of hospital stay increased by a median of 3 days in patients having healthcare-associated infections.[28]

Biofilm formation is one of the virulence factors responsible for CLABSI, with about half of the infections being caused by biofilm-producing bacteria. Our study found no correlation between clinical outcome and biofilm production, this contrasts with literature on biofilm formation and chronic wound infections. The persistence of chronic wounds beyond 14 days despite appropriate antimicrobial therapy has been linked to the formation of biofilms.[4] This could be because bloodstream infections are systemic and influenced by a wide variety of factors, unlike localized wounds. Nevertheless, biofilm-forming capability of microorganisms is an important factor leading to the colonization of intravascular devices and dissemination of infection.[29]

To the best of our knowledge, this is the first study trying to understand the role of biofilm production by the pathogens and their resistance pattern in progression and outcome of CLABSI. The study also endures a few limitations. First, the sample size was quite small, and to overcome the bias due to indications of long and complex surgeries requiring central line placement, and trauma; all the samples were collected from patients having medical indications of hospitalization. The bacterial isolates included were less due to the low culture positivity of CLABSI cases, which can be explained by the fact that patients usually receive various antibiotics before the admission. A bigger sample size might throw better light on understanding various factors affecting the course and outcome of CLABSI. Second, as biofilms are phenotypically and genotypically complex structures, understanding their functioning and pathogenicity requires broth dilution techniques and molecular methods.

Conclusion

The present study is the first one attempting to comprehend the interplay of various host and pathogen factors which affect the course and outcome of CLABSI. Studies with larger sample size and including patients from wards and ICUs of different departments such as surgery and trauma, and more detailed analyses will help in improved understanding of this complex interaction. The study has paved way for looking into details of one of the modifiable virulence factors-biofilm formation attributes of the bacteria. Future research directed on time taken for, and the role of specific drugs for prevention of biofilm formation, as well as specific drugs acting on Gram-positive and Gram-negative bacterial biofilms can be done for direct translation to the benefit of patients.

Research quality and ethics statement

This study was approved by the Institutional Ethics Committee (IECPG-498/17.07.2019). The authors followed applicable EQUATOR Network guidelines, i.e. STROBE Statement: Guidelines during the conduct of this research project.

Financial support and sponsorship

This study was financially supported by internal support from All India Institute of Medical Sciences, New Delhi, India.

Conflicts of interest

There are no conflicts of interest.

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