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Beyond the Surface: The Role of Biofilm Production and Antibiotic Resistance Pattern among Microbial Isolates from Neonatal Peripheral IV Catheter Samples in a Tertiary Care Hospital

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KEYWORDS

Neonates, Intravenous catheters, Biofilm, Antimicrobial resistance, NICU

ABSTRACT:

Background: Catheter-related infections (CRIs), particularly in vulnerable populations such as neonates, represent a significant clinical challenge due to the pervasive issue of biofilm formation and associated antimicrobial resistance. This study investigated biofilm-forming ability of microorganisms causing CRIs by analysing samples from intravascular catheters and blood. Our findings reveal a high rate of positive tip cultures, with 46.98% of peripheral intravascular catheters (IVC) showing colonization. Candida species were the most common organisms colonizing IVCs (70.7%), predominantly non-C. albicans (86.2%), followed by Coagulase-negative staphylococci (CoNS) (20.7%). A critical finding was the exceptionally high rate of biofilm production (95.1%) among clinical isolates, with 87.18% classified as strong biofilm producers. Antibiotic susceptibility testing demonstrated significant resistance patterns: all CoNS isolates were 100% resistant to Ampicillin and 88.2% resistant to Methicillin, yet were highly sensitive to Vancomycin (94.1%) and Linezolid (100%). Every gram-negative isolate was 100% dependent on imipenem but 100% resistant to ampicillin or amoxycillin-clavulanic acid. These results underscore the need to detect biofilm production in CRIs, given their persistent nature and significant antimicrobial resistance, highlighting crucial clinical implications for targeted management and prevention strategies, particularly in neonatal intensive care units (NICUs).

INTRODUCTION

Medical devices, particularly indwelling catheters, serve as prime surfaces for microbial colonisation and subsequent biofilm formation, an essential component of human microbial infections.[1,2] Biofilms are complex microbial communities attached to surfaces, which enhance pathogen virulence and pose substantial clinical challenges, "including impaired wound healing, chronic inflammation, recalcitrance to host immune defenses, quickly developed antibiotic resistance and the rapid spread of infectious emboli.[1,2,3,4] Implanted medical devices are thought to be responsible for 60–70% of nosocomial infections".[5] According to CDC or NIH reports, biofilm-caused infections occur among 65%-80% of the time.[2] Health-care-associated infections (HAIs) are a significant concern, with BSIs (bloodstream infections), UTIs (urinary tract infections), surgical site infections, or pneumonia accounting for a significant percentage.[6]

Catheter-related infections (CRIs) are particularly concerning in vulnerable populations, such as neonates, due to their immature immune systems and frequent need for invasive vascular access devices.[6,7] Understanding microbial profiles, biofilm-forming mechanisms, antibiotic resistance patterns, and prevention strategies is crucial to improving patient outcomes in this high-risk population.[6,7,8,9] The research aimed to examine biofilm-forming capability of microorganisms resulting CRIs, analyzing samples from intravascular catheters and blood.

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MATERIALS AND METHODS

This research was performed in the Department of Microbiology to assess the biofilm-forming capacity of organisms that cause CRIs. Clinical samples have been obtained from catheterized patients of both genders, encompassing intravascular catheters and associated blood samples from 166 individuals with proper informed consent obtained.

The study comprised inpatients who were catheterized for over 48 hours and displayed clinical signs of sepsis. Catheterization for less than 48 hours or those without sepsis symptoms were not included. A thorough clinical history was taken, including the length of the catheterization, swelling, fever with or without chills, and pain at the catheter site.

The intravenous catheter (IVC) site was assessed for swelling, erythema, local temperatures, and tenderness, followed by cleaning with alcohol. Sterile forceps were used to remove the catheter, ensuring that the externalized part remained away from the skin. The whole length of short catheters (< 6 cm) was aseptically cut 1cm below the surface/catheter junction. Two 5 cm segments (tip and intracutaneous segment) were obtained using lengthy catheters. Segments were transported in sterile, dry containers.[10]

Blood samples (5ml) were drawn using standard aseptic precautions, and inoculated into blood culture bottles.[11] Catheters were cultured utilizing semiquantitative method described via Maki *et al.*.. The entire catheter segment was rolled four times across a 5% sheep blood agar plate. Plates have been incubated at 37°C for 48hrs. Growth of >15 colonies indicated infection, while 1-14 colonies indicated contamination. Only samples with >15 colonies were included for further study. All colony types have been characterized using standard microbiological approaches. Catheter segments were "inoculated into 5ml of trypticase soy broth (TSB) and incubated overnight at 37°C; thereafter, they were subcultured onto Blood agar and MacConkey agar for" colony enumeration and identification.[12]

Catheter segments were Gram-stained after removing any clotted blood, air-drying, and longitudinally cutting opaque catheters. Gram staining was conducted using a series of petri plates with Lugol's iodine, Crystal violet, or dilute carbol fuchsin, followed by examination under oil immersion.[13]

The standard methodology was followed to identify each isolate by biochemical reactions. The Kirby-Bauer disc diffusion method has been used to evaluate Mueller-Hinton agar for antibiotic susceptibility.

Biofilm development was evidenced via Microtitre Plate Assay (Tissue Culture Plate Assay). The "isolates from new agar plates were diluted 1:100 in fresh medium after being inoculated into 5ml of TSB with 1% glucose and cultivated for 18hrs at 37°C. Sterile 96-well flat-bottom tissue culture plates made of polystyrene were filled with 0.2ml aliquots of diluted cultures, and broth-only controls were added for sterility verification. Following an 18-hour incubation at 37°C, the contents of the wells have been eliminated, and wells were rinsed four times with 0.2ml of phosphate-buffered saline (PBS, pH 7.2) to eliminate free-floating organisms. Adherent biofilms were preserved using 2% sodium acetate and subsequently stained with 0.1% crystal violet. Excess dye was removed, plates were dried, and optical density (OD) of" the stained adhering bacteria has been quantified utilizing spectrophotometer at 570nm. OD values below 0.12 signify weak or no biofilm production, values among 0.12 or 0.24 denote moderate biofilm production, while values over 0.24 indicate robust biofilm production.[14]

RESULTS

Our study collected 166 catheter samples in total, consisting of 105 males, 61 females, male-to-female ratio of 1.7:1. Microbial Positive tip cultures were found in 46.98% of peripheral intravascular catheters (IVC). A total of 82 isolates were obtained from IVCs. The commonest organisms colonizing IVCs were Candida species (58 isolates, 70.7%), with 50 (86.2%) being non-*C. albicans*. Coagulase-negative staphylococci (CoNS) followed at 17 isolates (20.7%), and *Klebsiella spp.* at 5 isolates (6.1%).

Biofilm Formation, a critical finding of our study, is the exceptionally high rate of biofilm production among clinical isolates, with 78 of 82 (95.1%) isolates being biofilm producers. Based on optical density values, 68 (87.17%) were identified as strong biofilm producers, 7 (8.97%) were moderate, and 3 (3.84%) were weak biofilm producers in our study. (Figure 1)

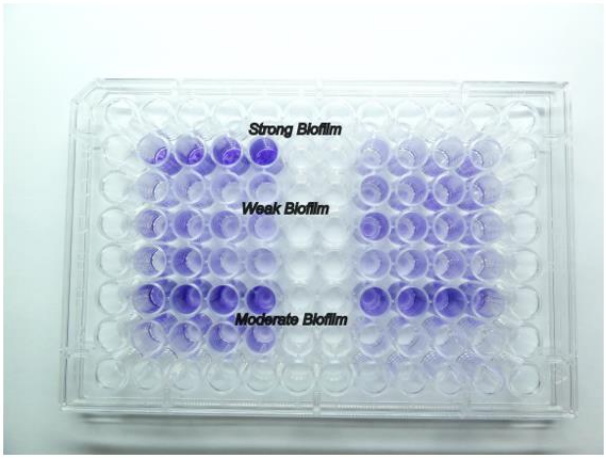

Figure 1 Biofilm production among the clinical isolates by Microtitre Plate Assay

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The antibiotic susceptibility testing revealed notable resistance patterns:

1. Coagulase-negative staphylococci (CoNS): All 17 isolates were 100% resistant to Ampicillin, and 88.2% were resistant to Methicillin. These isolates did, however, exhibit high sensitivity to several necessary antibiotics: 94.1% showed sensitivity to Vancomycin, while 100% demonstrated sensitivity to Linezolid. (Figure 2)
2. Gram-negative isolates: All 7 isolates were 100% reliant on imipenem but 100% resistant to ampicillin or amoxycillin-clavulanic acid. (Figure 3)

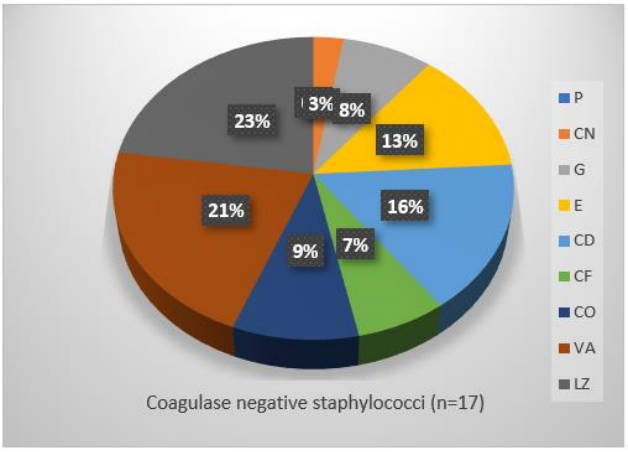

Figure 2: Antibiotic susceptibility pattern of biofilm producing Coagulase-negative staphylococci

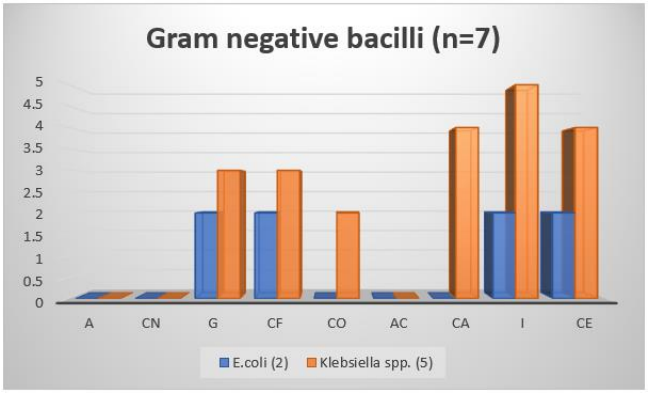

Figure 3: Antibiotic susceptibility pattern of biofilm producing gram negative bacilli

DISCUSSION

This study provides valuable insights into the microbial profiles, biofilm prevalence, and antibiotic resistance patterns of microorganisms causing CRIs, with a significant focus on samples obtained from neonates, a particularly vulnerable population.

Our findings on the prevalence of colonization for peripheral intravascular catheters (46.98%) correlate well with other studies, such as "Subha Rao *et al.* (52.5%) & Shaimaa *et al.* (43.3%). The incidence of local complications associated with peripheral intravenous catheters in neonates" is a well-documented concern.[7]

Regarding predominant pathogens, our study revealed a distinct profile. While our findings showed Candida species as the commonest organisms colonizing IVCs (70.7%), the broader literature, particularly concerning neonatal intensive care units (NICUs), consistently identifies CoNS as the most prevalent pathogens in NICU catheter infections, accounting for 64-84.2% of bacterial isolates from intravenous catheters.

Our high incidence of Candida species in IVC colonization is noteworthy, as fungal infections, particularly those caused by Candida species, are a growing concern in NICU settings, accounting for 42.11% of catheter-related BSIs in some NICU populations, with *Candida parapsilosis* often being predominant.[15] This high prevalence is attributed to factors like prolonged antibiotic use, parenteral nutrition, and invasive devices. *Klebsiella pneumoniae* is also recognized as a significant pathogen in "central line-associated bloodstream infections (CLABSI) or catheter-related bloodstream infections (CRBSI) in" NICUs aligning with its presence in our isolates.[15,16]

A critical finding of our study is the exceptionally high rate of biofilm production among clinical isolates, with 96.2% and 87.18% being found to be strong biofilm producers. This rate correlates well with findings from other studies, such as s Sangita *et al.* (88.8%) and Singhai *et al*. (81.5%). Systematic reviews indicate that 59-100% of clinical isolates from CRIs demonstrate biofilm formation capability, with 81.4% of colonizing isolates from peripheral intravascular catheters showing this ability.[5] Indwelling catheters create an artificial surface conducive to biofilm formation, increasing pathogen virulence and causing 60-70% of infections related to healthcare are due to implanted medical devices. Our results support the need to identify biofilm formation in CRIs because these infections are difficult to treat, frequently become persistent, and show high levels of antibiotic resistance.

Our study's antibiotic susceptibility testing revealed notable resistance patterns consistent with broader literature. All 17 CoNS isolates were 100% resistant to Ampicillin, with 88.2% also resistant to Methicillin. This aligns with studies reporting high prevalence of methicillin-resistant CoNS (MRCoNS) (up to 70%). However, our findings of high sensitivity to Vancomycin (94.1%) and Linezolid (100%) are consistent with

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studies reporting high susceptibility of CoNS to netilmicin, linezolid, and vancomycin.[2] For gram-negative isolates, all 7 were 100% resistant to Ampicillin or Amoxycillin-clavulanic acid, but 100% sensitive to Imipenem. This pattern also correlates with other studies. The literature further notes that *Klebsiella pneumoniae* isolates from NICU settings frequently exhibit ESBL (extended-spectrum beta-lactamase) production and multidrug resistance. The strong relationship among biofilm formation and multidrug resistance, highlighted in literature (OR=9.5), underscores why biofilm-producing microorganisms often have lower overall susceptibility to antimicrobials.[4]

Clinical Implications and Prevention Strategies

The high prevalence of biofilm-forming organisms and their associated antibiotic resistance emphasizes the significant clinical implications for managing CRIs. The necessity to detect biofilm production in CRIs is paramount due to their persistent nature and difficulty in eradication. Clinical guidelines suggest that empirical antibiotic regimens for CoNS infections should provide adequate coverage, considering the high rates of methicillin resistance.[17]

Prevention strategies highlighted in the literature include implementing maximal aseptic barriers during insertion, maintaining appropriate site and hub care, and recognizing catheter duration as a critical risk factor. Emerging strategies involve engineering catheter surfaces with optimized characteristics, such as appropriate oxygen levels, to minimize biofilm formation, and utilizing antimicrobial-impregnated catheters. Comprehensive bundle approaches, incorporating chlorhexidine skin antisepsis, sterile barriers, proper hand hygiene, optimal site selection, daily review of catheter requirement, have proven effective in reduction of CRIs. These prevention efforts are crucial given that certain high-risk populations, such as neonates with low birth weights or gestational ages and those requiring prolonged catheterization, are particularly vulnerable.[15]

CONCLUSION

Our research emphasizes significant role of biofilm formation in CRIs, with high proportion of isolates (96.2%) demonstrating this capacity and exhibiting considerable antimicrobial resistance. Our findings indicate that CoNS exhibited 88.2% resistance to methicillin and 100% resistance to ampicillin, while demonstrating 94.1% sensitivity “to vancomycin or 100% sensitivity to linezolid. Gram-negative bacteria exhibited complete resistance to Ampicillin or Amoxicillin-clavulanic acid”, while showing total sensitivity to Imipenem. These findings align with and contribute to the broader understanding of catheter-associated infections, highlighting the ongoing challenge posed by biofilms and the critical need for their detection and targeted management to improve patient outcomes.

Conflict of interest: Nil

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