**Original Research Article**

# A study on bacterial colonisation and biofilm formation on intravascular devices in Fathima Inistitute of Medical Sciences, Kadapa

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## Abstract

**Introduction:** Intra venous therapy for infusion of blood products, fluid and electrolytes, parenteral medications, hemodynamic monitoring, and for total parenteral nutrition (TPN) has become an essential feature of modern medical care. However, this is associated with the significant risk of infection of blood stream.

**Material and methods:** Intravenous cannulae with complaint of shooting pain and tenderness associated with cannulae were chosen as subjects of study. Tips of the cannulae were cultured using the semiquantitative method described by Maki.

**Results:** Among them, 32 (32%) cannulae were infected. Among the blood samples of these patients cultured, 14 (14%) were positive and 86 (86%) blood samples were sterile. Fourteen were Coagulase negative Staphylococci, ten were Coagulase positive Staphylococci and five were Micrococci. The others were Corynebacterium jeikeium (4), Candida parapilosis (2), Candida glabrata (2), Klebsiella pneumonia (2) and Pseudomonas aeruginosa (2).

**Conclusion:** There is no significant increase in the percentage of positive cultures with increase in duration of intravascular stay of the cannulae, after 24 hours. Majority of isolates from both cannula and blood are Coagulase negative Staphylococci (34.14% and 35.75% respectively). Majority of blood culture isolates are sensitive to Vancomycin.

## Key words

Bacterial colonisation, Biofilm formation, Intravascular devices.

## Introduction

Intra venous therapy for infusion of blood products, fluid and electrolytes, parenteral medications, hemodynamic monitoring, and for total parenteral nutrition (TPN) has become an essential feature of modern medical care. However, this is associated with the significant risk of infection of blood stream. Short peripheral venous cannulae, usually inserted into the veins of the forearm or hand, remain the most commonly used intravascular devices [1, 3].

Since the emergence of antibiotic era, bacteria and antibiotics are on constant fight to outwit each other. The bacteria are rapidly developing mechanisms to resist the antibiotics, and newer antibiotics are discovered which can counteract the bacterial resistance [4, 5].

Among the varied mechanisms of antimicrobial resistance, formation of biofilms is a recently evolved and identified mechanism. Though Antony Van Leeuwenhoek observed them on tooth surface, their importance has been identified by only Prescott, as well as the concept of bacteria forming a group (similar to the unions and associations of human beings). They communicate to each other by chemical signals, facilitating an interactive and coordinated activity. Bacteria prefer a community-based, surface-bound, sedentary lifestyle to a nomadic existence [6-10].

The present study attempted to isolate bacteria forming biofilms on intravascular devices. The study was planned in the premises of Fathima Hospital, a tertiary care hospital with out-patient strength of more than 2000 per day, and an average inpatient admission of 300 - 400 per day. Most of the inpatients admitted require intra venous alimentation or therapy, and a substantial percentage of them develop fever after insertion of IV cannulae.

The objective of the present study was to isolate bacteria from biofilms formed by bacteria on the intravenous cannulae and their association with bacteremia and septicemia. Timely detection of this problem helps the clinicians in treatment.

## Materials and methods

The study period was from April to July 2014. After obtaining approval from the Institutional Ethics Committee, as many as 100 in patients who had intravenous cannulae with complaint of shooting pain and tenderness associated with cannulae were chosen as subjects of study. Written informed consent from these inpatients was obtained. The co morbid, immunosuppressed, diabetes mellitus, malnutrition, steroid therapy, malignancies or those on anti-malignancy drugs patients were completely excluded. The peripheral venous cannulae were collected from these patients when clinically indicated, using sterile precautions in the medical, surgical, neurosurgical, pediatric, and gynecological wards. The inpatient number, name, sex, age, date, presenting complaints, duration of symptoms, purpose of insertion of cannulae, date of insertion of cannula , history of fever after insertion of cannula and the unit in which the patient was admitted was noted. The intravenous portion (2-3 cm) of the cannula was cut with sterile scissors and placed in a sterile container and from the same patient 10 ml blood was collected from the peripheral vein with a sterile disposable syringe both of them were immediately transferred to the Microbiology laboratory. Catheters were cultured using the semiquantitative method. Flamed forceps were used to transfer the catheter segment onto the surface of a 5% sheep blood agar plate rolled over the blood agar plate four times. Simultaneously, 10 ml of venous blood was inoculated into bottle containing 100 ml of BHI

broth. The inoculated samples were incubated for 24 hours at $37^0$c. An uninoculated BHI broth and the blood agar plate acted as Negative Control.

After 24 hours of incubation, subculture was done on blood agar and Mac Conkey agar. The isolates from blood agar and Mac Conkey agar were identified by employing standard microbiological techniques. All isolates were maintained on brain heat infusion agar till they were subjected to the three methods for biofilm production, namely tissue culture method, tube method and congo red agar method.

## Results

**Number and percentage of positive cultures in the present study** (*P Value < 0.0001)

A total of hundred patients who were admitted into various units in Fathima Hospital, Kadapa were selected as subjects of the present study. All of them had indwelling intravenous cannulae, for diagnostic or therapeutic purpose. Cannulae were removed and cultured, from those who complained of pain and swelling at the site of cannulation. Among them, 32 (32%) cannulae were infected. Among the blood samples of these patients cultured, 14 (14%) were positive and 86 (86%) blood samples were sterile.

**Relation of culture positivity with pyrexia**

The relation of culture positivity with fever: Out of 32 patients with positive cannula cultures, 10 (31%) had pyrexia after cannulation, whereas out of 14 patients with positive blood cultures, 6 (42.8%) had fever after cannulation.

List and number of isolates from cannula culture was as per **Table - 1**. Fourteen isolates were Coagulase negative Staphylococci, ten were Coagulase positive Staphylococci and five were Micrococci. The others were Corynebacterium jeikeium (4), Candida parapilosis (2), Candida glabrata (2), Klebsiella pneumonia (2) and Pseudomonas aeruginosa (2).

**List and number of isolates from blood cultures**

Among the isolates, 5 (35.75%) were Coagulase negative Staphylococci, 6 (42.85%) were Coagulase positive Staphylococci and 3 (21.42%) were Micrococci.

**Table - 1:** List and number of isolates from cannula cultures.

| Name of the Organism | Number isolated |
|---|---|
| Coagulase negative Staphylococci | 14 |
| Coagulase positive Staphylococci | 10 |
| Micrococci | 5 |
| Corynebacterium jeikeium | 4 |
| Candida parapsilosis | 2 |
| Candida glabrata | 2 |
| Klebsiella pneumonia | 2 |
| Pseudomonas aeruginosa | 2 |

**Colonisation of bacteria on cannulae in relation to the duration of intravascular stay**

Nine cannulae were removed after 24 hours, and are processed. Among them, 4 (44%) were found to be infected. Simultaneously, blood cultures from the same patients were sterile. 18 cannulae were processed after 48 hours among them, 6 (33 %) were positive for cannula culture and 1 (5%) was positive for blood culture. 27 cannulae were processed after 72 hours of intravascular stay among them, 12 (44.4%) were positive for cannula culture and 5 (18.5%) were positive for blood culture. Out of 46 cannulae processed after 96 hrs of intravascular stay, 19 (41%) were positive for the cannula culture and 8 (19.5%) were positive for blood culture.

**Distribution of samples according to the clinical condition**

Out of 30 samples collected from patients suffering from gastrointestinal disorders, 16 (53%) cannulae and 4 (13%) blood samples were positive. 24 samples were collected from patients suffering from central nervous system disorders among them, 6 (25%) each of cannulae and blood showed positive culture. 15 samples were

collected from patients suffering from musculoskeletal disorders and among them, 7 (46%) cannulae were positive for culture and all samples of blood from the same patients were sterile. Among 14 samples processed from patients suffering from bronchopulmonary disorders, 3 (21%) cannulae and 2 (14.2%) samples of blood were positive.

Five samples were processed from patients of disorders of renal system among them, 1 (20%) was positive for cannula culture and all blood samples from the same group were sterile. Among 2 patients suffering from ENT disorders, 1 (50%) sample was positive for cannula culture and both blood cultures were negative. Among 3 samples collected from patients with genitourinary infections, 2 (66%) cannulae and 2 (66%) blood samples were positive. Six samples were collected from patients suffering from fever, and among them 4 (66%) were positive for cannula culture, and all blood samples were sterile. 3 samples were processed from patients suffering from cardiovascular disorders, 1 (33%) showed cannula culture positive, and all the three blood samples were sterile.

**Distribution of samples according to age**

Among a total of 100 samples, 10 were from patients less than 20 years. Among them, 5 (50%) were positive for the cannula culture and 1 (10%) was positive for blood culture. 39 samples belonged to the age group between 21 to 40 years. Among them, 16 (41%) were positive for cannula culture and 4 (10%) were positive for blood culture. 51 samples were collected from patients belonging to the age group of more than 40 years. In this group, 20 (39%) were positive for cannula culture and 9 (17.6%) were positive for blood sample culture.

Evaluation of techniques used to detect biofilm production was as per **Table - 2**. Out of 41 isolates from cannulae, tissue culture plate method showed 22 high grade positives, 14 moderate positives and 5 weak or non biofilm producers. The tube method showed 18 high positives, 14 moderate positives and 9 weak or non biofilm producers. Congo red agar method showed 8 high positives, 12 moderate positives and 21 weak or non biofilm producers.

Out of 14 isolates from blood cultures, tissue culture plate method showed 6 high positives, 5 moderate positives and 3 weak or non biofilm producers. The tube method showed 2 high positives, 2 moderate positives and 10 weak or non biofilm producers. Congo red agar method showed 1 high positive, 3 moderate positives and 10 weak or non biofilm producers.

**Table - 2:** Evaluation of techniques used to detect biofilm production.

| Grading of the Biofilm | Tissue Culture Plate Method | | Tube method | | Congo Red Agar Method | |
|---|---|---|---|---|---|---|
| | Cannula culture | Blood culture | Cannula culture | Blood culture | Cannula culture | Blood culture |
| **High** | 22 | 6 | 18 | 2 | 8 | 1 |
| **Moderate** | 14 | 5 | 14 | 2 | 12 | 3 |
| **Weak/ Non Biofilm Producers** | 5 | 3 | 9 | 10 | 21 | 10 |

**Association of bacteremia with positive cannula cultures**

Out of 100 samples each, 9 (9%) were found to be positive for both cannula culture and blood culture. 32 (32%) were found to be positive for cannula culture and negative for blood culture. 5 (5%) were found to be negative for cannula culture but the blood culture was positive. 52(52%) were negative for both cannula and blood culture.

**Antibiogram of blood culture isolates of Coagulase Positive Staphylococci**

Antibiogram of blood culture isolates of Coagulase positive Staphylococci, out of 6 CPS 5 (83%) sensitive to Vancomycin and 1 (17%) is resistant - the zone diameter 17 – 21 mm was taken for evaluation of resistance. Three (30%) were sensitive to Oxacillin and 3 (50%) resistant - the zone diameter 18 - 24mm was taken for evaluation of resistance. Two (33%) were sensitive to Penicillin G and 4 (67%) were resistant - the zone diameter 26 - 37mm was taken for evaluation of resistance. Three (50%) were sensitive to Tetracycline and 3 (50%) were resistant - the zone diameter 24 – 30 mm was taken for evaluation of resistance. One (17%) sensitive to Gentamycin and 5 (83%) were resistant – the zone diameter being 19 – 27 mm and all (100%) were resistant to Cotrimoxazole and Cefotaxime - the zone diameter 24 – 32 mm and 25 – 31 mm was taken for evaluation of resistance.

**Antibiogram of blood culture isolates of Coagulase negative Staphylococci**

Antibiogram of blood culture isolates of Coagulase negative Staphylococci, out of 5 CNS all (100%) sensitive to Vancomycin zone diameter of 17 - 21mm was taken for evaluation of resistance. Three (60%) were sensitive to Oxacillin and 2 (40%) resistant the zone diameter 17 – 21 mm was taken for evaluation of resistance. All of them were resistant to Penicillin G, Co-trimoxazole, Cefotaxime and 1 (20%) sensitive to Gentamycin and 5 (80%) were resistant zone diameter being 19 – 27 mm.

**Percentage of slime positive and slime negative isolates from cannula and percentage of slime positive and slime negative isolates from blood**

Out of 41 positive cannula cultures, 14 isolates were Coagulase negative Staphylococci among which 13 were slime producing and 1 was non slime producing. Ten isolates were Coagulase negative Staphylococci, 13 were slime producing and 1 non slime producing. Among 17 isolates which were other than Staphylococci, 13 were slime positive and 4 were slime negative.

Out of 14 positive blood cultures, 5 were Coagulase negative Staphylococci and 4 strains produced slime. Six isolates were Coagulase negative Staphylococci, 4 strains produced slime. Among 3 of other isolates, one strain produced slime.

## Discussion

The use of intravascular devices, both venous and arterial, to deliver sterile fluids, medications and nutritional products, as well as for central monitoring of hemodynamic functions has dramatically increased during the past decade. Because catheters inserted into the venous/ arterial blood stream by-pass the normal defense mechanisms [11-13]. These devices provide a way for micro-organisms to enter into the body at the time of insertion through procedural defects, or the devices are subsequently contaminated by colonizing organisms. Comparative evaluation of techniques used to detect slime production was as per **Table – 3** [4, 5, 14].

## Conclusion

Among 100 catheter tip specimens cultured, 41 were positive (41%). Among 100 blood samples cultured, 14 were positive (14%). Majority of samples were from gastrointestinal ward (30%). Majority of isolates from both cannula and blood were Coagulase negative Staphylococci (34.14% and 35.75% respectively). There was no significant increase in the percentage of positive cultures with increase in duration of intravascular stay of the cannula, after 24 hours. There was a significant increase in the incidence of cannula associated bacteremia in persons over 40 years. TCP method was evaluated as most sensitive among the 3 methods used to detect slime production. Maximum number of Coagulase negative Staphylococcal isolates were slime positive (92.85% among cannula isolates and 62.66% among blood isolates). Majority of blood culture isolates were sensitive to Vancomycin.

**Table - 3:** Comparative evaluation of techniques used to detect slime production.

| Year | Author | Method | Positivity |
|---|---|---|---|
| 1985 | Christensen, et al. [14] | TCP | 90.6% |
| 1988 | Zufferey, et al. | AO | 94% |
| 1999 | Ammendolia, et al. [4] | TCP | 63.2% |
| 2001 | Donlan, et al. | SEM | 63% |
| 2011 | Arciola, et al. [5] | PCR | 49% |
| 2014 | Present study | TCP | 89.47% |

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