



**ORIGINAL RESEARCH PAPER**

**Clinical Microbiology**

**DEMONSTRATION OF BIOFILM FORMATION BY GRAM-NEGATIVE BACTERIA AND STUDY THEIR AST PATTERN AT MGMMC, JAMSHEDPUR, JHARKHAND.**

**KEY WORDS:** Biofilm, gram-negative bacteria, hospital acquired infections (HAI), antibiotic resistance, antibiogram

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**ABSTRACT**

**Introduction:** Gram- negative bacteria are attributable to matrix- enclosed aggregates known as biofilms. The development of biofilms is currently recognised as one of the most relevant drivers of persistent infections and constitutes as a major challenge for clinical microbiologist and clinicians. Bacteria living in a biofilm can exhibit a 10 to 1,000-fold increase in antibiotic resistance compared to similar bacteria living in a planktonic state. **Aims & Objective:** The purpose of this study is to detect the biofilm formation by gram-negative bacilli and determine their antimicrobial susceptibility pattern. **Methods:** A cross-sectional study was conducted from January 2024 to November 2024 on 246 bacterial isolates from various clinical samples to demonstrate biofilm formation by Tube Method and an antibiogram was drawn. **Result:** Escherichia coli (34.15%) was the predominant gram-negative bacteria encountered, followed by Pseudomonas aeruginosa (28.05%), Klebsiella pneumoniae (22.36%) and Acinetobacter baumannii (10.16%). AST revealed higher resistance to Ampicillin, Trimethoprim/ sulfamethoxazole, Amoxicillin clavulanic acid, Gentamicin and Cefepime. **Conclusion:** Biofilms are rapidly becoming a major contributor to Hospital Acquired Infections (HAI) The knowledge of biofilm formation and antibiogram of bacterial isolates contributing to its formation is of utmost importance for rendering reliable empirical antibiotic therapy to the patients.

**INTRODUCTION**

A biofilm is a community of microorganisms, such as bacteria, that are capable of living and reproducing as a collective entity known as a colony. To put it another way, biofilms are living biomass that possess a sophisticated social structure that personnel involved in this field are still attempting to decipher. [1]

The structure of biofilm serves both to shield and enable the expansion of the colony. Biofilm formation is a multi-factorial and complex process. The formation of a biofilm begins when bacteria sense environmental stress conditions that trigger the bacterial life to adhere to the surface, either biotic or abiotic. The development cycle of biofilms contains complex and progressive processes which are divided into four stages: initial adhesion, formation of microcolonies, biofilm maturation, and detachment and dispersion. The process of biofilm formation is reversible in the initial stages and depends on environmental conditions but once the colonies pass the initial attachment and adhesion phase, it can lead to irreversible attachment to different surfaces. Later, genetic and phenotypic changes take place within the bacteria encapsulated in the matrix of biofilm and lead to biofilm-induced resistance. We divide these changes into physical mechanisms and biological mechanisms. Physically, bacteria produce a thick biofilm matrix to evade antimicrobial agents. [2]

Several studies on infectious diseases have pointed to an association with biofilms. A less virulent pathogen becomes more virulent, acquiring more antibacterial resistance when it is part of a biofilm. Genetic transfer in a biofilm community modifies the bacterial population, enhancing secretion of various secretory substances and changing cell signatures, which increases the bacterial resistance. Treatment failure is due to acquired resistance through various secretory substances and cell signatures. The spread of multi-drug resistant microorganisms found in biofilms is worsening situations all around the globe and emerging as a new threat

to public health [3].

Costerton et al. was the first to demonstrate an association between biofilm and medical devices. Conservative estimates suggest that roughly 70% of all bacterial infections are linked with biofilm and are both device-related and non-device-related [11].

Subsequent studies have demonstrated urinary catheters, central venous catheters, indwelling stents, contact lenses, intrauterine devices, and dental chair water lines all to be susceptible to bacterial adhesion and biofilm formation. [3]

The most encountered cause of infection due to bacterial biofilm is often associated with indwelling catheters [4]. Catheters used for less than 10 days have a high chance of biofilm formation on their external surfaces, while catheters used for over 30 days are more likely to develop biofilms in their lumens [5]. Bacteria that grow in catheters depend on the fluid compositions administered through the catheter [6]. The most common species are Gram-negative bacteria, such as P. aeruginosa, Enterobacter and Klebsiella species, which can easily grow in intravenous fluids [3]. Most commonly used catheter is the urinary catheter, which is inserted through the urethra into the bladder and is usually composed of latex or silicon, which favors bacterial attachment [7]. An open system catheter is more prone to bacterial contamination and, therefore, transfers of diseases, such as urinary tract infections (UTIs). E. coli, Enterococcus faecalis and Proteus mirabilis are among the most common pathogens in UTIs [7]. Contact lenses are also susceptible to bacterial biofilm growth because of the adhesive nature of both the lenses and the containers used to keep cleaning lens solution. Bacteria, such as E. coli, S. aureus, Serratia and Proteus, are responsible for biofilm formation in contact lenses and cause severe infections, such as keratitis. In addition, P. aeruginosa may lead to blindness following severe keratitis [8]. Other organisms, such as Streptococcus, Enterococcus and Candida, can form biofilms on mechanical heart valves and

cause endocarditis [9].

Beta-lactams were wonder drugs until the dissemination of beta-lactamases (ESBL and MBL) producing strains were detected. Extended-spectrum beta-lactamases (ESBLs) may be defined as plasmid-mediated enzymes that hydrolyze oxyimino-cephalosporins (ceftriaxone, cefotaxime, and ceftazidime) and monobactams (aztreonam) but not cephamycins or carbapenems [3]. Biofilm formation and beta-lactamases production synergistically contribute for extensive dissemination of multi-drug resistant strains of gram-negative bacilli. They are responsible for implicating chronicity, persistence, and relapse of infections leading to high morbidity and mortality; thus, posing a serious health crisis [10]. Hence, this study was conducted to detect the biofilm formation by gram-negative bacilli and determine their antimicrobial resistance pattern along with the detection of ESBL and MBL production.

**Aims & Objective:**

The purpose of this study is to detect the biofilm formation by gram-negative bacilli and determine their antimicrobial susceptibility pattern.

**MATERIALS & METHODS:**

This cross-sectional study was conducted at the Department of Microbiology in Mahatma Gandhi Memorial Medical College & Hospital, Jamshedpur, Jharkhand from January – October, 2024. 246 non-repetitive gram-negative isolates were recovered from the clinical specimens such as blood, urine, pus, CSF, endotracheal tube, tracheal aspirate, fluids, catheter tips, sputum, submitted to the microbiology lab for routine culture and sensitivity testing. The samples were received from various inpatients wards and outpatient departments of this hospital. Emphasis was given on specimens from patients with long standing duration of ICU stay. All specimens were inoculated on Blood and MacConkey agar except urine specimens which were plated on Cysteine Lactose Deficient Medium (CLED) as per the standard bacteriological procedures. The culture plates were incubated at 35 °C for 24–48 h. The growth isolates were identified on basis of colony morphology, pigmentation, odours, and their unique biochemical tests. Their AST pattern was studied by Kirby Bauer disc diffusion method. The multi-drug resistant isolates, esp. ESBL- strains, were selectively subjected to biofilm detection by Tube Method.

The isolates were then tested for biofilm formation by Tube Method (TM). Sterile Glass Test tubes were used. Bacterial suspensions were prepared in trypticase soy broth. The broth was then transferred to the sterile glass test tubes and incubated at 37°C for 20- 24 hrs. To demonstrate biofilm formation, the broth was discarded with all necessary biomedical waste measures and the now emptied glass tubes were first washed with phosphate buffer saline and treated with 0.1% crystal violet stain. Biofilm is visible as stain adherent to the bottom and wall of the test tubes.



**Fig.1 :** AST plate showing multi-drug resistant



**Fig.2:** Biofilm adherence to glass test tube is shown by staining with 0.1% crystal violet

**RESULT:**

Out of the 246 clinical samples submitted to microbiology lab, maximum clinical samples were urine followed by blood, pus, aspirates and sputum. As for bacterial isolates, Escherichia coli (34.15%) was the predominant gram-negative bacteria encountered, followed by Pseudomonas aeruginosa (28.05%), Klebsiella pneumoniae (22.36%) and Acinetobacter baumannii (10.16%). Other bacteria like Proteus spp., Enterobacter, Citrobacter accounted for remaining 5.28% (Table.1) The Antimicrobial susceptibility test for the bacterial isolates revealed higher resistance to Ampicillin, Trimethoprim/ sulfamethoxazole, Amoxicillin clavulanic acid, Gentamicin and Cefepime. The isolates, however, remained sensitive to Imipenem.

**Table:1. No. of Bacterial isolates in various clinical samples**

| CLINICAL SAMPLES            |                         | Urin e | Bloo d | Pus | Aspir ates | Sput um |
|-----------------------------|-------------------------|--------|--------|-----|------------|---------|
| No. of BACT ERIAL ISOL ATES | Escherichia coli        | 72     | 7      | 3   | 2          | 0       |
|                             | Pseudomonas aeruginosa  | 28     | 18     | 14  | 6          | 3       |
|                             | Klebsiella pneumoniae   | 26     | 12     | 2   | 3          | 12      |
|                             | Acinetobacter baumannii | 5      | 14     | 5   | 1          | 0       |
|                             | Proteus                 | 2      | 4      | 2   | 0          | 0       |
|                             | Enterobacter            | 1      | 1      | 2   | 0          | 0       |
|                             | Citrobacter             | 0      | 1      | 0   | 0          | 0       |

**DISCUSSION:**

Diseases caused by microbial biofilms are serious public health issues because of the increasing antimicrobial resistance. It has been demonstrated that bacteria in biofilms are a thousand times more resistant to antibacterial drugs compared to those in the planktonic state.

An article published in 2022 has found that among Gram-negative bacteria, E. coli (38%) is a predominant clinical isolate that forms biofilms, along with Acinetobacter species (20%), Klebsiella species (16%) and Pseudomonas species (12%) [1]. It is in concordance with our study.

The other notable results of our study are corroborated by a similar study done by Damru et.al BPKIMS in 2019 [12].

The bacterial colony in biofilm ecosystem is under tremendous stress. This facilitates horizontal gene transfer among the bacterial to survive in the stressful conditions. Drug resistant gene exchange, like that of ESBL, has been documented to occur between the biofilm microbiome. Also, the evolving resistance caused by extended-spectrum - lactamases (ESBLs) led to higher morbidity, prolonged hospital stays, and expensive treatment options.

As antimicrobial stewardship and infection prevention programs continue to evolve, it will be increasingly important to understand the dangers posed by biofilms, and how

preventing the transfer and acquisition of biofilm-causing organisms can directly impact the spread of AMR.

**CONCLUSIONS:**

Given the persistence of biofilms in the hospital setting, and their ability to create an ideal environment for resistance mechanism exchange, greater awareness of these dangers is needed. The notable prevalence of biofilm-forming and multi-drug resistant organisms provides a glimpse of the upcoming threat in our part of the world. The simple yet efficient Tube Method of biofilm demonstration enables to detect biofilm forming bacteria even in simpler laboratory set ups. Our study positively states that implementation of routine monitoring of biofilm in clinical laboratories will significantly help not only in treating the patient but also preventing hospital acquired infections.

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