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A STUDY ON BIOFILM FORMATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA IN WOUND INFECTION

Ms. Shravana Kumari[1], Dr. Sweetha Nayak Nagar[2], Dr. Likith N.[3], Dr. Vijaya[4]

[1] Tutor, Department of Microbiology, Srinivas Institute of Medical Sciences, Mukka, Mangalore, Karnataka, India.
[2] Associate Professor, Department of Microbiology, Srinivas Institute of Medical Sciences and Research Centre, Mukka, Mangalore, Karnataka, India.
[3] MBBS Intern, Srinivas Institute of Medical Sciences and Research Centre, Mukka, Mangalore, Karnataka, India.
[4] Professor, Department of Microbiology, Srinivas Institute of Medical Sciences and Research Centre, Mukka, Mangalore, Karnataka, India.

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Corresponding Author:

Dr. Sweetha Nayak Nagar
Associate Professor, Department of Microbiology, Srinivas Institute of Medical Sciences and Research Centre, Mukka, Mangalore, Karnataka, India.

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ABSTRACT

Background; As the human body's largest organ, the skin is vital for life, maintaining water and electrolyte balance, regulating temperature, and serving as a barrier against harmful external agents and microorganisms. A wound occurs when this epithelial integrity is breached, leaving the body vulnerable to infections caused by diverse pathogens, including bacteria, fungi, parasites, and viruses. While common bacterial culprits include Staphylococcus aureus, Klebsiella pneumoniae, E. coli, Proteus, Acinetobacter species and Pseudomonas aeruginosa, has become a significant concern. Recent data shows a marked increase in P. aeruginosa wound infections compared to studies from a decade ago, manifesting in both acute and chronic forms. Furthermore, it is estimated that 28% of healthy individuals within hospital environments are carriers of this specific Gram-negative bacilli.
Method; This is a cross sectional study to determine the biofilm formation and Antibiotic susceptibility pattern (AST) of Pseudomonas aeruginosa in wound infection. The study was conducted between January 2025 to December 2025 in Microbiology laboratory at Srinivas institute of Medical sciences and Research centre, Mukka Hospital, Mangalore.
Result; Out of 41 isolates of P. aeruginosa, it showed maximum resistance to cephalosporins and least resistance to carbapenems. Out of 41 P. aeruginosa isolates, 16 were biofilm producers, of which 8, 6, and 2 were weak, Moderate, and strong biofilm producers respectively. Of the total 16 biofilm producing P. aeruginosa isolates, 4 were Metallo-β Lactamase (MBL) producers and 12 were MBL non producers.
Conclusion; This study showed that Pseudomonas aeruginosa is the most common organism isolated from wound infections and that the presence of MBL producing Pseudomonas aeruginosa is alarmingly increasing in wound infections.

Keywords: *Wound infection, Pseudomonas aeruginosa, Biofilm production, MBL producing organisms, and Gram negative bacteria.*

INTRODUCTION

Being the largest organ of the human body, the skin is vital for survival as it maintains fluid and electrolyte balance, regulates body temperature, and serves as a protective shield against harmful external agents such as microorganisms. When its epithelial integrity is breached, a wound occurs. The progression to an infected state is likely influenced by various microbial and host factors, including wound characteristics, host general health, and microbial virulence. Wound infections can be caused by a range of microorganisms, with common bacterial culprits including *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli, Proteus, and Acinetobacter spp*. Recently, the Gram-negative bacilli *Pseudomonas aeruginosa* has gained significant attention due to its increased incidence in wound infections and its role as a key pathogen in both acute and chronic infections; notably, 28% of healthy individuals in hospital settings have been observed to be carriers.[1,2]

A critical element in the pathogenesis of lethal *P. aeruginosa* infections is the pathogen's ability to develop biofilms on both living tissues and inert surfaces. Unlike their free forms, bacteria within biofilms exhibit increased resistance to antibiotic treatments and immune system responses, frequently resulting in persistent, deep-seated infections. These structures are stabilized by an extracellular polymeric matrix produced by the bacteria; within this matrix, polysaccharides serve as vital structural components that help to shield the bacteria from antimicrobial agents.[3] In addition to their inherent drug resistance, the capacity of these microorganisms to develop biofilms effectively neutralizes immune responses and antibiotic therapies. This biofilm serves as a protective shield, safeguarding the bacteria from host defenses and obstructing the delivery of medication, which can ultimately lead to impaired wound healing.[4,5]

To combat pathogenic bacteria capable of biofilm formation, three primary non-microbicidal strategies have been proposed: preventing initial surface attachment, disrupting the biofilm's architecture to facilitate antimicrobial penetration, and interfering with maturation to induce dispersal or degradation. These approaches are particularly relevant to *Pseudomonas aeruginosa*, a pathogen that poses a severe threat to human health due to its environmental versatility and multi-drug resistance. A central component of this pathogen's resilience is the development of *P. aeruginosa* biofilms, which progress through several distinct and specialized growth stages.[6,7]

Pseudomonas aeruginosa is frequently associated with biofilm formation and the production of MBLs, both of which are primary drivers of severe clinical infections. The global dissemination of acquired MBLs among critical pathogens, including *P. aeruginosa* and members of the Enterobacteriaceae family, has become a major public health crisis. Patients infected with MBL-producing strains face significantly higher mortality rates, often due to the failure of initial treatments. These enzymes are particularly dangerous because they hydrolyze nearly all beta-lactam antibiotics, with the sole exception of monobactams. Early detection is vital to prevent their spread within and between healthcare facilities. This proactive screening is essential even in institutions where these resistant phenotypes have not yet been identified.[8,9,10]

OBJECTIVES

This study mainly focuses on isolating *Pseudomonas aeruginosa* isolates from wound infection and to know their antibiotic suscebtibility pattern and to check the biofilm formation and MBL production.

MATERIALS AND METHODS

This cross sectional study was conducted between January 2025 to December 2025 at Srinivas institute of Medical sciences and Research centre, Mukka Hospital, Mangalore. *Pseudomonas aeruginosa* isolates from wound infection were selected for the study. The isolates were subjected to antibiotic sensitivity testing by Kirby-Bauer Disk Diffusion method and interpretation was made as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[11]

Microtiter plate assay was performed to determine the biofilm formation. A single colony of test organism was inoculated in 2mL Brain Heart Infusion Broth (BHIB) in the test tube and incubated at 37° C for overnight. 200 µl from each of the inoculated BHIB tubes was aseptically transferred in the wells of a sterile, flat bottomed, polystyrene microtiter plate. Those microtiter plates were further incubated at 37° C for 24 hours without sealing of the plate for proper oxygenation. After incubation, the contents of the well were discarded by inverting the plate and striking it on filter paper. The microtiter wells were washed once by adding 200 µl PBS into each well and then discarded. 200µg of freshly prepared 2% sodium acetate was added to each well (for biofilm14 fixation) for 10 minutes and then discarded. This was followed by adding 200 µl of 0.1% crystal violet to each well for 30 minutes at room temperature. Excess stain was discarded. Washing step was repeated again. The plates were left to dry at room temperature and the optical densities were determined by spectrophotometer reader at wavelength of 620nm.[12]

Mueller-Hinton agar was used for Modified Hodge Test (MHT). 10µg Meropenem disc was placed at the centre of the plate and the test strains were streaked from the edge of the disc to the periphery of the plate and the plates were incubated over-night at 37ºC and organisms producing Cloverleaf shaped zone of inhibition were identified as MBL producers and interpreted as MHT positive.[13]

RESULTS

A total of 41 *P. aeruginosa* strains from the wound samples were included in this study. Out of 41 isolates highest resistance was exhibited to cephalosporins and least to carbapenems and aminoglycosides. Out of 41 *P. aeruginosa* isolates, 16 were biofilm producers, of which 8, 6, and 2 were weak, Moderate, and strong biofilm producers respectively. Of the total 16 biofilm producing *P. aeruginosa* isolates, 4 were MBL producers and 12 were MBL non producers.

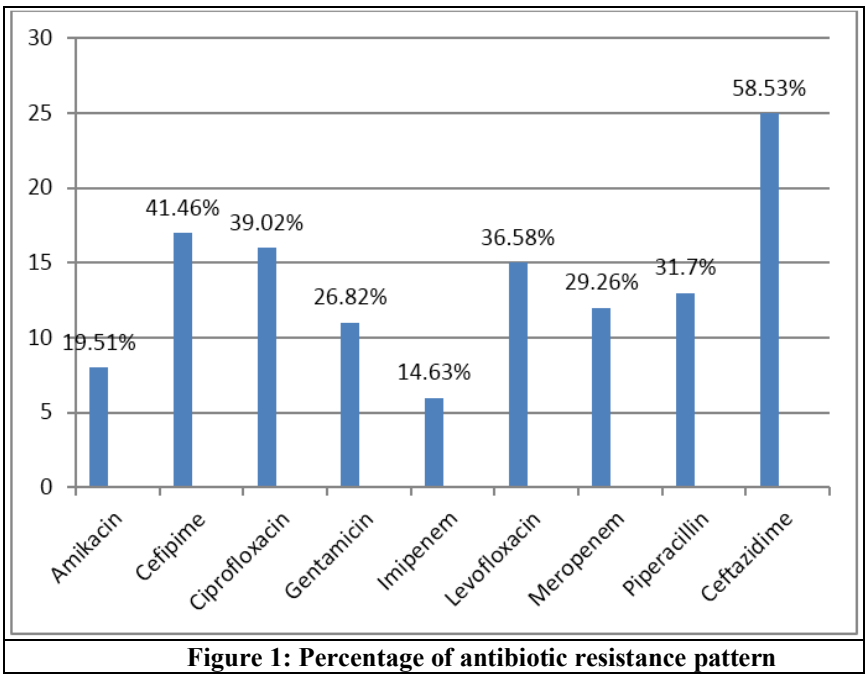

Figure 1: Percentage of antibiotic resistance pattern

Table 1: Shows Biofilm formation by P. aeruginosa isolate

Total No. of Isolates	Number of *P.aeruginosa isolates* producing Biofilm			
41	No biofilm	Strong	Moderate	Weak
	25	2	6	8

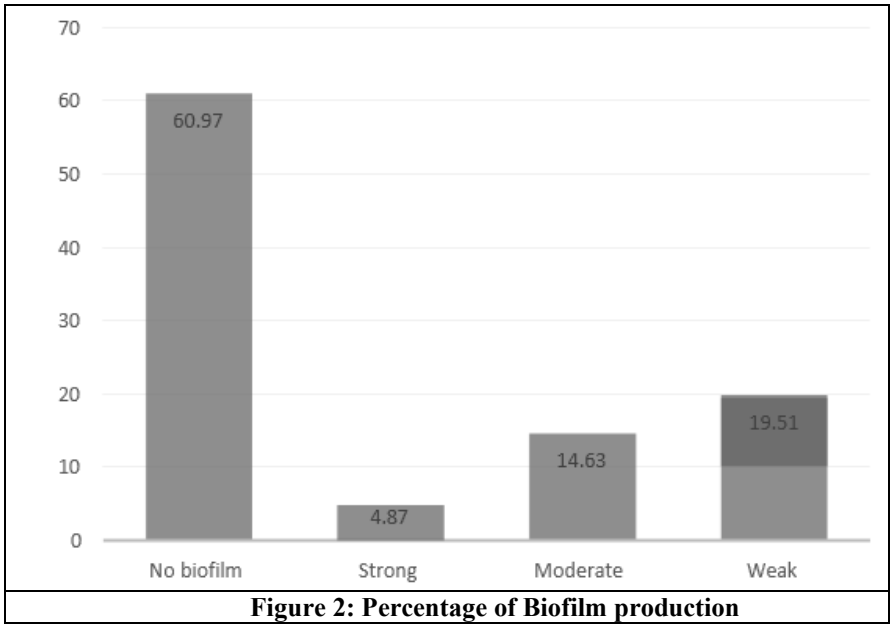

Figure 2: Percentage of Biofilm production

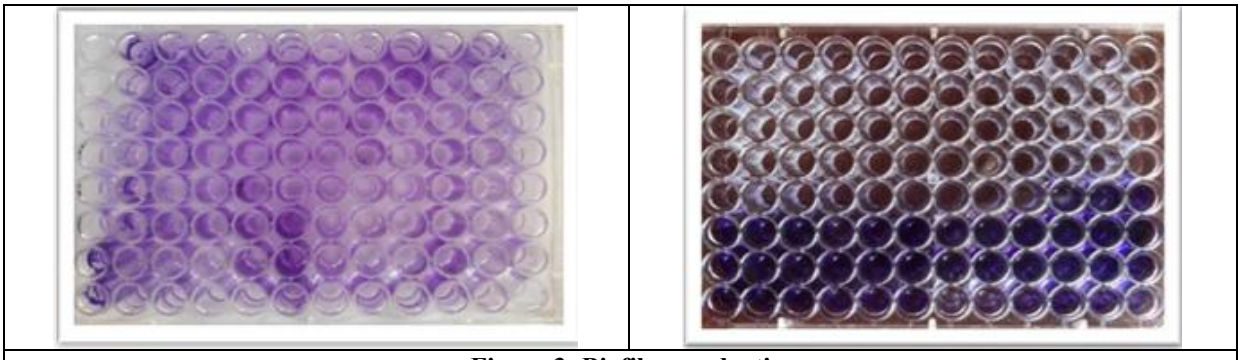

Figure 3: Biofilm production

Table 2: Production of MBL by biofilm producing P. aeruginosas

Total no of Biofilm Producers	MBL Producers	MBL Non Producers
16	4	12

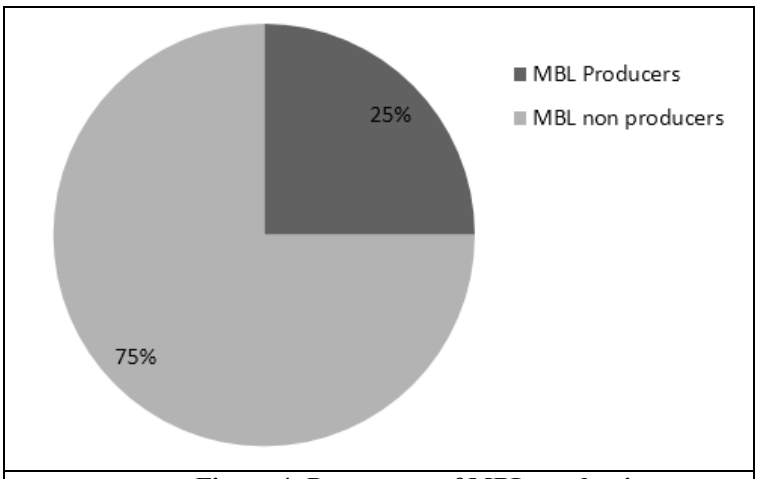

Figure 4: Percentage of MBL production

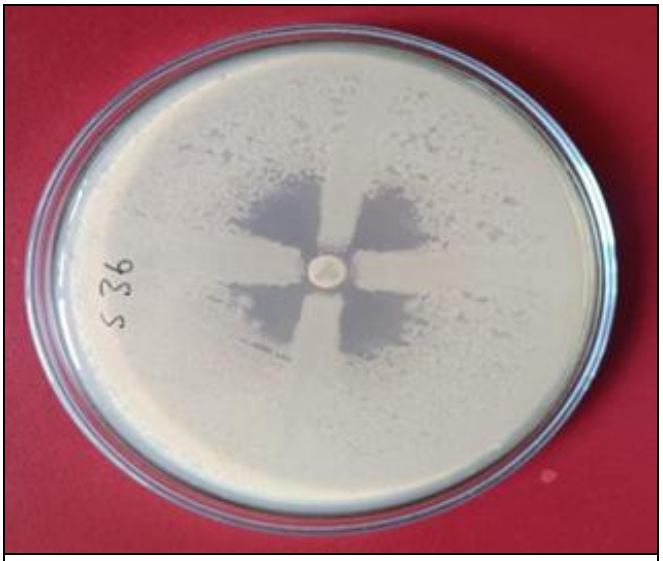

Figure 5: MBL production

DISCUSSION

Pseudomonas spp is a large group of aerobic, non sporing, Gram-negative bacilli, motile by polar flagella. They cause infections in chronic wounds often forming protective biofilms, secreting toxins and hindering healing by disrupting tissue repair and immune responses.

Wound infection affects 1-2% of the general population and is related to increased morbidity and health costs. *Pseudomonas aeruginosa* is the most common bacteria isolated from the wounds. They can express virulence factors and surface proteins affecting wound healing.[14]

The present cross sectional study consists of 41 wound samples, where it showed highest resistance to cephalosporins and least to carbapenems and aminoglycosides. These findings were similar to the study conducted by Breiyeh Z et al.[15]

Out of 41 *P. aeruginosa isolates,* 16 were biofilm producers, of which 8 (19.52%) were weak, 6 (14.63%) were moderate and 2 (4.87%) were strong biofilm producers. A similar study was conducted by Samira Heydari,et al, where it showed more percentage of strong biofilms production which was contradictory to the present study.[3]

Of 16 biofilm producing *Pseudomonas aeruginosa isolates*, 25 % were MBL producers and 75% were MBL non producers. Other similar studies like Hala B. Othmanetal, showed more percentage of MBL producers than MBL non producers.[16]

CONCLUSION

Pseudomonas aeruginosa is one of the important organisms causing wound infections, which has the ability to form biofilms. Formation of biofilm is an important patho physiological step in wound infections which prolongs the duration of wound healing. It plays main role in the disease progression and chronicity of the lesion, the development of resistance and makes wound healing difficult to treat.In this study biofilm producing isolates showed MBL production which increases the morbidity.

These observations are important especially for patient management and better outcome.

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