

# Biofilm Producing *Pseudomonas Aeruginosa* Isolates From Infected Wound: A Demagogue for Clinicians

[Dr Harman Multani](#), Dr Varsha A Singh, Dr. Aishiya Ishrat, Dr. Seema Aleem, Abhishek Kumar Paul, Dr. Sonia Mehta, Dr. Shinu Pottathil, Dr. Shivya J Thakur

Corresponding Author : Dr. Harman Multani

Address: Maharishi Markandeshwar University, MMIMSR

Microbiology Department

Mullana

Ambala, Pincode : 133207(Haryana, India)

Email: [multanih14@gmail.com](mailto:multanih14@gmail.com)

## ABSTRACT

**Background:** Infected wounds has become a colossal problem in clinical and public health setting. *Pseudomonas aeruginosa* is the worst pathogens of wound due to its versatility in virulence and multi drug resistance ability for the community as well as hospital settings. Many studies have predicated the role of biofilm as an inhibitor of wound healing as it greatly combats body immune systems and antimicrobials. *Pseudomonas aeruginosa* in hospitalized patients is the epitome of delayed wound healing. The present study was aimed to detect biofilm formation by Multi Drug Resistant *Pseudomonas aeruginosa* strains resistant to Cefepime, Ciprofloxacin and Amikacin(MDR) from

wound infections ,early treatment can be planned.

**Methods:** *Pseudomonas aeruginosa* strains resistant to Cefepime, Ciprofloxacin and Amikacin from Wound/Pus samples from clinically suspected patients with Wound infections were subjected to biofilm formation by three phenotypic methods viz Tissue Culture Plate Method, Tube Adherence Method and Modified Congo Red Agar Method.

**Results:** out of total 150 wound specimens 75(50%) isolates were *Pseudomonas aeruginosa*. of which 60(79.5%) were MDR. and 36(60%) isolates of MDR *Pseudomonas aeruginosa* showed Biofilm

formation .MDR *Pseudomonas aeruginosa* was substantially associated with Diabetes foot infection (84.2%) with p value=.0001 was statistically significant. Meropenem came out to be most sensitive drug against biofilm producing MDR *Pseudomonas aeruginosa*.

**Conclusion:** *Pseudomonas aeruginosa* is prone to biofilm formation and not to ignore especially in diabetes. You must suspect biofilm formation if they are resistant to Cefepime, Ciprofloxacin and Amikacin. Meropenem comes to be most sensitive so every hospital should form antibiotic policy for wound infections.

**Key Words:** Biofilms, *Pseudomonas aeruginosa*, Infected wounds

## INTRODUCTION

Human skin wound has become a colossal problem in clinical and public health setting. Wounds once colonized and infection being established it becomes a snowballing threat. *Pseudomonas aeruginosa* is one of the worst pathogens of wound due to its versatility in virulence for the community as well as hospital settings. Multi-Drug resistant *Pseudomonas aeruginosa* in hospitalized patients is the epitome of delayed wound

healing .. Since chronic ,non healing wounds pose a serious clinical problems for instance necrosis, marjolin's ulcers and even a fatal outcome like septicaemia . Furthermore, many studies have predicated the role of biofilm as an inhibitor of wound healing as it greatly combats body immune systems and antimicrobials. Each amassment of bacteria creates a unique biofilm with different characteristics so that a clinical approach has to be altered to the specifics of a given biofilm. This leads to further detection of Biofilm causing non-healing chronic infections which embraces the future aspect of various chronic diseases. Therefore the present study was aimed to detect biofilm formation by Multi Drug Resistant *Pseudomonas aeruginosa* strains [which acquired as non-susceptibility to at least one agent in three or more antimicrobial categories(carbapenems, fluoroquinolones, penicillins/cephalosporins and aminoglycosides].i.e resistant to Cefepime, Ciprofloxacin and Amikacin(MDR) from wound infections so that early treatment can be planned.

## MATERIAL & METHOD

The study was conducted in the department of Microbiology,MMIMSR,Mullana,Amb

ala. Ethical clearance was taken from the ethical committee. A total of 60 Wound/Pus isolates of *Pseudomonas aeruginosa* strains resistant to Cefepime, Ciprofloxacin and Amikacin from the clinically suspected patients with Wound infections attending (OPD) and (IPD) were detected and subjected to Biofilm formation by - **Tissue Culture plate method ,Tube Adherence method and Modified Congo red agar method.**

## RESULTS

A total of 150 wound specimens were processed, predominant 75(50%) isolates were *Pseudomonas aeruginosa*. Out of these strains 60(79.5%) isolates were MDR..Interestingly 36(60%) isolates of MDR *Pseudomonas aeruginosa* showed Biofilm formation(Table I) MDR *Pseudomonas aeruginosa* was substantially associated with Diabetes foot infection (84.2%) followed by Burn wound infection (64.7%) , Site of prosthetic wound infection (60%),Venous leg ulcer ( 57.1%), Bed sore (50%) and osteomyelitis (40%). (Table II) All the MDR *Pseudomonas aeruginosa* strains which were isolated in the present series were tested against various antibiotics.

Meropenem came out to be the most sensitive drug against biofilm producing MDR *Pseudomonas aeruginosa*. The other two most sensitive drugs were Imipenem (69.44%) and Cefoperazone-sulbactam (66.66%) with Piperacillin/ Tazobactam.

## DISCUSSION

Biofilm production is a way through which bacteria combat the effect of antibiotics and it makes the bacteria almost impossible to be eradicated. Off late, biofilm is getting produced at an alarming rate further enhancing the need to be detected and treated. The critical factors for the survival of *Pseudomonas aeruginosa* in the unfavorable environment is its ability to transform from the “Swarm cell” to glycocalyx enclosed micro colony which serves to protect against the active phagocytes, enzymes and high level of specific antibodies. The positivity rate of biofilm producing pathogenic *Pseudomonas aeruginosa* in **the current study**, came out to be 60% which was well in accordance with the studies done by **Zubair M et al (2011)<sup>1</sup>** and **Nagaveni S. et al (2010)<sup>2</sup>** in which *Pseudomonas aeruginosa* as biofilm producers have come out to be 52.1% .The higher rate of biofilm production may be

because of selection criteria of patients.

### (TABLE I)

Wound infections is known to impair wound healing with the immunocompromised patients. The microorganism that colonizes the surface wound provide an ideal niche for further invasion resulting in infection. **In the existent study**, among the various associated condition rate of biofilm production showed the highest positivity with Diabetics foot infection (84.2%) followed by Burn wound infection with 64.7% positivity, Site of prosthetic wound infection with 60% positivity, Venous leg ulcer with 57.1% positivity and osteomyelitis with 40% of positivity and with p value =0.0001, which is highly significant. It was very much supported by **Swarna SR. et al (2012)<sup>3</sup>**, where biofilm production on Diabetics foot ulcer patient was 100% as due to various other isolates was taken. Besides that, in case of Burn, it was shown of 68.75% of biofilm production by *Pseudomonas aeruginosa*, supported by **Hadi Mohammad Huda (2013)<sup>4</sup>**. It was due to the increased number of immunocompromised patients in this particular study. In the case of Prosthetic implant related biofilm formation due to cellular adhesion on

metals demonstrated a linear correlation with surface energy and surface energy may be a more important determinant of cell adhesion and proliferation, and may be more useful than surface roughness for directing cell adhesion and cell colonization which facilitates microorganisms to proliferate and undergo phenotypic alteration which leads to biofilm development which was supported by **Stoodley P et al (2005)<sup>5</sup>** and **Hallab NJ et al (2001)<sup>6</sup>**. All the above conditions are allied to chronic conditions. Biofilm play an essential role in wound chronicity, as per microscopic evaluation of specimens from the chronic wounds often indicates the presence of biofilm, which is supported by **Martínez-Pastor Juan carlos et al (2013)<sup>7</sup>**. In the other **conditions which** were not shown very well biofilm production because of the acute clinical conditions. **(Table II)**. The Antibiotic resistant bacteria are bacteria that are not swayed or killed by antibiotics. They are able to sustain themselves and even procreate in the presence of an antibiotic.. Bacteria that are resistant to many antibiotics are known as multi-resistant organisms. Most disappointing fact about biofilm forming bacteria is their ability to be resistant to most of the drugs. The current

study has included *Pseudomonas aeruginosa* strains already resistant to Amikacin, Ciprofloxacin and Cefepime. This makes it essential to look for the drugs which should effectively upon such bacteria. The most sensitive drugs against biofilm forming pathogenic *Pseudomonas aeruginosa*, in the present study were Meropenem (75%) and Imipenem (69.44%). It is concerning with the study done by Hassan Afreenish et al (2011)<sup>8</sup> and Zaranza Alicia Valeria et al (2013)<sup>9</sup> in which the above mentioned drugs were most sensitive for the biofilm forming pathogenic gram negative bacteria. This is probably due to the fact that Meropenem and imipenem along with other chosen drugs in the study like Colistin, Cefoperazone-Sulbactam and Polymyxin-B are not commonly administered in the wound infection as compared with Cefepime, Ciprofloxacin and Amikacin as those are non-traditional antibiotics, as the use of following is uncertain due to their toxic effects. (Table III)

**Conclusion:** To conclude, in the present study rate of biofilm formation in Wound infection came out to be significantly high 60% at least to an extent which should not be ignored anyway. Hence, whenever encounter multi-drug resistant (Amikacin,

Ciprofloxacin and Cefepime) *Pseudomonas aeruginosa*, subject it for detection of biofilm formation. In Diabetes mellitus patient (84.2%), they are very much prone to biofilm formation so such patients should also be screening for biofilm detection. The multi-drug resistant biofilm producing *Pseudomonas aeruginosa* are usually sensitive to Meropenem and Imipenem.

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**TABLE I: RATE OF BIOFILM PRODUCTION WITH MULTIDRUG RESISTANT PSEUDOMONAS AERUGINOSA**

TOTAL NUMBER OF MDR PSEUDOMONAS AERUGINOSA	BIOFILM PRODUCTION (OVERALL POSITIVITY BY ANY OF THREE METHODS)
60	36 (60%)

**TABLE II: CORRELATION OF ASSOCIATED CONDITIONS WITH BIOFILM FORMING PSEUDOMONAS AERUGINOSA**

ASSOCIATED CONDITION	TOTAL NO. OF SAMPLES N=150	RESISTANT PSEUDOMONAS AERUGINOSA N=60	BIOFILM PRODUCER N=36
Post-operative Wound	36	3	0 (0%)
Diabetes foot ulcer	26	19	16(84.2%)
Venous leg ulcer	12	4	2(57.1%)
Burn wound infection	21	17	11(64.7%)
Prosthetic wound Infection	10	5	3(60%)
Osteomyelitis	10	5	2(40%)
Bed sore	10	04	2(50%)
Otitis Media	10	0	0(0%)
Abscess	15	3	0(0%)

Chi-square ( $\chi^2$ ) value = 45.174, p-value = 0.0001, As the p value is less than the level of significance i.e. 0.05 so the result is "Significant"

TABLE III: ANTIBIOTIC SENSITIVITY PATTERN OF BIOFILM PRODUCING PSEUDOMONAS AERUGINOSA

ANTIMICROBIAL AGENTS	PERCENTAGE (%) (N=36)
Meropenem	27(75%)
Imipenem	25(69.44%)
Cefoperazone-Sulbactam	24(66.66%)
Piperacillin/ Tazobatam	23(63.88%)