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# Isolation and Characterization of Pseudomonas Aeruginosa Bacteriophages

**Jangili Pavan Kumar[1], Shaik Muzammil Pasha[2], Yemgdda Goutham Sudhan[3], Chand Pasha[4]**

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**Abstract**

Pseudomonas aeruginosa is a notorious, opportunistic pathogen and has ability of biofilm formation making it possible to survive in extreme conditions. As antibiotics and disinfectants have shown limited activity, P. aeruginosa phage based treatments are proposed as a promising way for biofilm control. P. aeruginosa isolates and PB1 like phage particles were isolated from sewage samples collected from sewage treatment plants. PB1 like phage was featured with large burst size of 151PFU/cell, host inactivation in 6 hrs, 80% of host range and had icosahedral head with a diameter of 85X80nm and long non contractile tail of 135nm. PB1 like phages are suitable for lysis of broad range MDR Pseudomonas biofilms, which could be used in phage therapy.

**Keywords:** Pseudomonas aeruginosa; Bacteriophages; PB1; Cell lysis; Biofilm.

## INTRODUCTION

Antibiotics play an important role as therapeutic and prophylactic in healthcare clinics, veterinary, agriculture, food processing industries, etc. However, indiscriminate uses of antibiotics have led to development of resistance to most of the antibiotics (Reygaert, W. C., 2018). Additionally, the decline in research and development of new antibiotics has limited choice of weapons against pathogenic bacteria (Nathan, C, 2020). It is assumed that, by 2050, antibiotic resistance will result in 10 million deaths (Bassetti, M. *et al.*, 2017). In recent years, increasing morbidity and mortality due to infections with multidrug resistant pathogens have become a serious concern (De Oliveira et al., 2020). Because of the rapidly increasing cases of antimicrobial resistance, India too has recently declared K. pneumoniae, E. coli, A. baumannii, and P. aeruginosa as the 'critical priority' pathogens (DBT, 2021). The phage therapy through administration of lytic bacteriophages has proved successful in the war against these pathogens (Schooley *et al.*, 2017, Duplessis *et al.*, 2018, Qin *et al.*, 2021, Petrovic Fabijan *et al.*, 2020, Patil *et al.*, 2021, El Haddad *et al.*, 2019, Mulani *et al.*, 2019). Among the resistant pathogens, P. aeruginosa is a gram negative opportunistic pathogen, predominantly found in hospitals, animal farms, slaughter houses, soil, aquatic environment, and sewage water. P. aeruginosa is notorious for being the major cause of death by nosocomial infections,

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especially in patients with severe wounds, causing sepsis in immune suppressed patients, chronic lung infections in patients with cystic fibrosis, and chronic obstructive lung disease, bladder catheter associated chronic infections in the urinary tract and ventilator associated serious pneumonia (Diggle *et al.*,2020). In most cases, treatment of P. aeruginosa is very challenging owing to its multiple mechanisms to resist antibiotics and ability to form antibiotic resistant bioflms (Ciofu *et al.*, 2019).

P. aeruginosa is a biofilm forming with multiple antibiotic resistances and degradation of various molecules (Hall-Stoodley *et al.* 2004; Harrison *et al.* 2008; Kumari *et al.*, 2009). P. aeruginosa is a principle microbe responsible for nosocomial infections (Driscoll JA *et al.*, 2007, Hancock RE & Speert DP, 2000). Phage therapy is a great option in combating P. aeruginosa infections (Fu *et al.*, 2010, Wright A *et al.*, 2009, Marza *et al.*, 2006). The infectiveness of antibacterial component, intervention of biofilm brought a Steve growth of phages as an alternative to prevent biofilm formation. Despite of several people reported phages of P. aeruginosa isolation, characterization but utilization for lysis of biofilm are limited. Here we described the characterization of PB1 phage and its possible exploring to block biofilm.

## MATERIALS AND METHODS

Source of bacteria: P. aeruginosa was isolated from samples collected from sewage treatment plant, Necklace road Hyderabad, Telangana, India.

Serial dilutions of the sewage water were prepared, inoculated on LB Agar containing Citramide Plates and incubated at 37°C for 24 - 48 h. A single colony was transferred onto slant surface of Nutrient agar and incubated at 37°C for 48 h. The bacterial isolates were identified by growth on specific media, microscopy, arginine dehydrolase test, coagulase test, catalase test, oxidase test, H2S test and mannitol fermentation test.

**Antibiotic susceptibility test:**

Antibiotic susceptibility of Isolated P. aeruginosa strains was determined by Kirby Bauer Disc diffusion method. Eight different antibiotics were used in these tests are chloramphenicol, gentamicin, azithromycin, levofloxacin, doxycycline, ciprofloxacin, tobramycin and amikacin.

**Isolation of the bacteriophages:**

*Bacteriophage enrichment:*

Bacteriophage enrichment was done by taking 4ml of 0.2 µ filtered sample water suspension (phage source), 1ml of 10x Luria broth and 1ml of exponential growing P. aeruginosa and incubated at 37°C for 24 hr. Then the suspension was centrifuged at 15000 rpm for 5 mins and filtered through 0.2 µ syringe filter. The filtrate was mixed with pure culture and over layed using double agar layered based plaque assay method.

**Detection of bacteriophages / Plaque assay:**

In a sterile eppendorf, 0.2 µ syringe filtered 100 µl of bacteriophage source and 100 µl of exponential bacterial culture were added and incubated at 37°C for 15 min, then it was mixed with 7ml low melting agar (0.8%) and poured onto nutrient agar plate. Allowed the low melting agar to solidify for 30 min at room temperature and then plates were incubated inverted at 37°C for 24h.

**Isolation of Pure phage:**

Using a sterile scalpel, an isolated plaque was picked from the overlayed nutrient agar plate and suspended in 500 µl of phage buffer and diluted. A dilution was mixed with exponential bacterial culture; incubated and under gone double agar layered based plaque assay. Individual plaque obtained in this method was selected.

**Host Inactivation studies:**

Pure P. aeruginosa strains were inoculated separately into different flasks containing nutrient broth and incubated at 37°C for 24hr. Then the flasks were infected with 0.2µ filtered phages and incubated at 37°C with gentle shaking. The sample was collected from different flasks for every 1 hour, till 8 hours consecutively. The hourly samples of flasks were spread on the nutrient agar plates respectively for viability of host cells. The numbers of colonies in the hourly samples were counted by using colony counter. The time required to kill 90% of initial cells was measured.

**Burst size determination:**

An isolated plaque was picked into a sterile eppendorf containing 500 µl of phage buffer and then it was added to 500 µl of bacterial culture in a eppendorf and 100 µl of the mixture was undergone double layered agar based plaque assay.

**Phage purification:**

*Centrifugation:*

Phage lysate was made cell free by centrifuging at 5000rpm for 10 min and clear lysate was again centrifuged at 15000rpm for 5h to precipitate phages. The pellet was suspended in the phage buffer.

**PEG:**

By centrifugation of 15000rpm for 5min, the cells

were removed and the supernatant was collected. PEG 8000 was added to the supernatant solution to make 2% concentration and stirred at 4°C overnight to precipitate the bacteriophages. Then the solution was centrifuged at 15000 rpm for 10 mins, bacteriophages were collected as pellet and suspended in phage buffer and dialyzed.

**Transmission electron microscopy of phages:**

One drop of the purified phage suspension was placed on a copper grid with carbon coated Formvar film for 10 mins at room temperature. 4% aqueous phosphotungstic acid was used for staining at pH 7. The sample was air dried overnight and examined with a Zeiss TEM 900 electron microscope; it was operated at 50 kV. The phage particles were visualized using the Image SP software and a CCD camera.

**Determination of host range:**

The host range of obtained phages was determined by P. aeruginosa strains. 1ml of pure P. aeruginosa strains, were spread on nutrient agar plates respectively. 50 µl of phages were sprayed on the nutrient agar plates with pure culture. These plates were incubated at 37°C for 24 hrs. Then plates were observed for plaques.

**Biofilm formation studies:**

For the quantification of biofilm formed, overnight cultures of P. aeruginosa, was diluted to 106 CFU/ml into fresh LB broth supplemented with 1% glucose. 100µl of culture was diluted with 100µl of the same medium and added to 800µl of LB broth placed into each well of 24 well plates. The media changed for every 12 hrs without shaking. Half the volume of old medium in the culture was replaced with fresh medium. The development of biofilms was monitored up to 96 hrs. Biofilms formed were fixed with 200µl of methanol for 15 mins, followed by the addition of crystal violet and incubated for 15 mins. The wells were then washed with water and dried for 2 hrs at room temperature. 200µl of ethanol (95%) was added to dissolve the stain. The absorbance of eluted stain was measured at 570nm in a spectrophotometer. The absorbance at 570nm is a direct indication of the amount of biofilm formed. The biomass was represented as O.D. at 570nm.

**Bacteriophage treatments:**

In this study, the ability of bacteriophages to act on biofilms was determined. To accomplish this, biofilms formed by P. aeruginosa, after 12, 24, 36, 48, 60, 72, 84 and 96 hrs were infected with P. aeruginosa phage. About $1 \times 10^9$ phage particles suspended in 0.5 mL were added to the different aged biofilms and incubated for 4 hrs, immediately after incubation with the phages, the biofilms were quantitated by staining with crystal violet and measuring the absorbance at 570 nm.

**Statistical analysis:**

Experiments were repeated thrice in triplicates (n = 9) and average values with standard deviation was provided.

## RESULTS

*Isolation and identification of P. aeruginosa:*

Five strains of P. aeruginosa were isolated from sewage samples, collected from sewage treatment plants. P. aeruginosa were identified by growth on Citramide agar, microscopy, Biochemical characteristics and the results were presented in Table 1.

Five strains were identified as P. aeruginosa based as white colonies on Citramide agar. They were gram negative, rod shaped and 1-3 x 0.4-0.7 µm in size. In the biochemical tests, it was Arginine dehydrolase postive, coagulase negative, catalase

**Table 1:** Identification of P.aeruginsa by growth on specific media, microscopic morphology and biochemical tests.

| Strain | Growth on specific media | Microscopy morphology | Biochemical Tests | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Arginine dehydrolase test | coagulase test | catalase test | Oxidase test | H2S test | Mannitol fermentation test |
| P. aeruginosa 1 | white colonies on Citramide agar | Gram negative, rod shaped and 1-3 x 0.4-0.7 µm in size. | positive | Negative | positive | positive | Negative | Positive |
| P. aeruginosa 2 | white colonies on Citramide agar | Gram negative, rod shaped and 1-3 x 0.4-0.7 µm in size. | positive | Negative | positive | positive | Negative | Positive |
| P. aeruginosa 3 | white colonies on Citramide agar | Gram negative, rod shaped and 1-3 x 0.4-0.7 µm in size. | positive | Negative | positive | positive | Negative | Positive |

*table cont.....*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| P. aeruginosa 4 | white colonies on Citramide agar | Gram negative, rod shaped and 1-3 x 0.4-0.7 µm in size. | positive | Negative | positive | positive | Negative | Positive |
| P. aeruginosa 5 | white colonies on Citramide agar | Gram negative, rod shaped and 1-3 x 0.4-0.7 µm in size. | positive | Negative | positive | positive | Negative | positive |

positive, oxidase positive, H2S negative and mannitol fermentation positive.

**Antibiotic susceptibility test:**

Results showed that P. aeruginosa was resistant to majority of antibiotics. High rate of resistance was against doxycycline, azithromycin, chloramphenicol and ciprofloxacin. It was intermediately susceptible to gentamycin, levofloxacin, tobramycin and amikacin. It was not highly susceptible to any of the antibiotics.

**Table 2:** Antibiotic susceptibility test of P. aeruginosa as zone of inbition (mm) with 100µ concentration.

| Bacteria | Strain | Chloram-phenicol | Gentamicin | Azithro-mycin | Levo-floxicin | Doxy-cycline | Cipro-floxacin | Tobra-mycin | Amikacin |
|---|---|---|---|---|---|---|---|---|---|
| P. aeruginosa 1 | HN1 | 7.04 | 10.24 | 2.85 | 11.45 | 2.56 | 8.3 | 10.28 | 15.36 |
| P. aeruginosa 2 | HN2 | 7.01 | 10.11 | 2.24 | 11.25 | 2.21 | 7.6 | 10.19 | 15.12 |
| P. aeruginosa 3 | HN3 | 6.98 | 9.98 | 2.48 | 10.89 | 1.99 | 8.1 | 10.54 | 14.89 |
| P. aeruginosa 4 | HN4 | 7.03 | 10.02 | 2.59 | 11.56 | 2.24 | 7.9 | 10.98 | 15.19 |
| P. aeruginosa 5 | HN5 | 7.01 | 10.22 | 2.78 | 11.94 | 2.17 | 7.3 | 10.33 | 14.94 |

**Bacteriophage enrichment:**

Phage enrichment filtrate contained numerous phages and formed plaques of varying sizes specific to P. aeruginosa strains.

**Detection of bacteriophages/plaque assay:**

In Plaque assay, after incubation, bacteriophage plaque formation was determined and plaques were counted as plaque forming units (PFU). P. aeruginosa plaques were round as in fig. 1.

**Fig. 1:** Plaque assay of P. aeruginosa showing plaques

**Phage purification:**

Plaque was purified and used in plaque assay method which produced plaques specific to P. aeruginosa on nutrient agar plates. Single and isolated plaque was selected for pure phage.

**Host Inactivation Studies:**

The number of colonies in hourly samples were counted using colony counter. The viable cell count was more till 1 hour, from 2nd hours, the number of viable cells started decreasing in a descending order. 90% of P. aeruginosa cells were inactivated in 6 hours.

**Burst size determination:**

Plaques were observed on the nutrient agar plates. The plaque with largest burst size was the bacteriophage with higher affectivity. P. aeruginosa phage produced the burst size of 151 PFU per cell.

**Bacteriophage isolation and purification:**

The P. aeruginosa bacteriophages were isolated from sewage samples, phages were like lambda phages in morphological appearance, having icosahedral head with a diameter of 85 x 80nm and long non contractile tail of 135 nm based on TEM. It is PB1 like phage in Fig. 2.

**Determination of Host range:**

Host range was determined using five P.

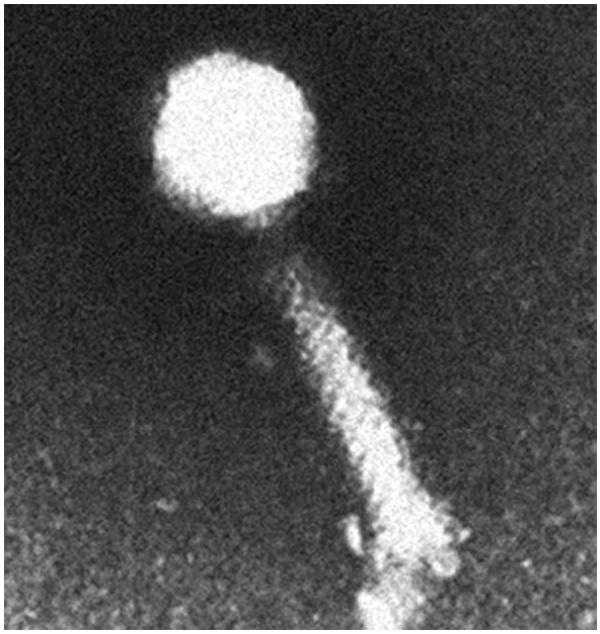
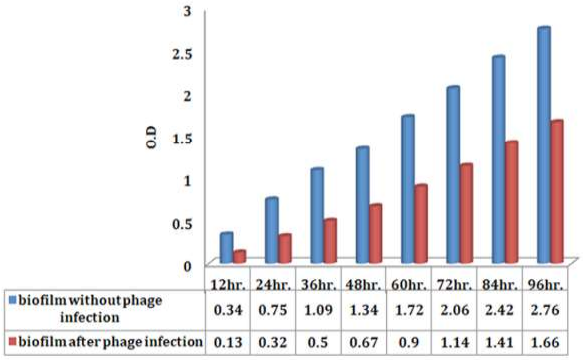


**Fig. 2:** Transmission electron microscopy image of P. aeruginsa phage.

aeruginosa strains. The P. aeruginosa bacteriophage had 80% of host range as it infected four out of five P. aeruginosa strains.

**Effect of phage treatment on the bacterial biofilms:**

P. aeruginosa biofilms obtained were treated with the phages for 2–4 hrs. Immediately after incubation with phage, the biofilms were stained by crystal violet assay. The phage reduced the biomass of P. aeruginosa bacterial biofilms in a time dependent manner. Upon phage treatment for 4 hrs the O.D. decreased in biofilms of 12h (0.34± 0.04 to 0.17± 0.05), 24 h (0.75 ± 0.03 to 0.38 ± 0.08),36h(1.09± 0.04 to 0.55± 0.06), 48 h (1.34 ± 0.01 to 0.72 ± 0.02), 60h(1.72± 0.02 to 0.91± 0.03), 72 h (2.06 ± 0.037 to 1.08 ± 0.017), 84h(2.42± 0.01 to 1.25± 0.04) and 96 h (2.76 ± 0.05 to 1.45 ± 0.026).




| | 12hr. | 24hr. | 36hr. | 48hr. | 60hr. | 72hr. | 84hr. | 96hr. |
|---|---|---|---|---|---|---|---|---|
| biofilm without phage infection | 0.34 | 0.75 | 1.09 | 1.34 | 1.72 | 2.06 | 2.42 | 2.76 |
| biofilm after phage infection | 0.13 | 0.32 | 0.5 | 0.67 | 0.9 | 1.14 | 1.41 | 1.66 |

**Fig. 3:** Impact of P. aeruginosa phage on the biofilms by crystal violet staining.

## DISCUSSION

Pseudomonas form biofilm on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems have become problematic (Hall-Stoodley *et al.* 2004). Biofilms are difficult to deal than the single bacterial infections as they require high doses of antibiotics, longer periods of time for treatment. This leads to increase in antibiotic resistance among the microbes in biofilms. Biofilm formation leads to less penetration of antibiotics (Nair *et al.* 2016). Pseudomonas are prevalent in sewage (Piracha *et al.*, 2014), hospital (Remold SK *et al.*, 2011), lakes (ZehraChegini *et al.*, 2020), etc. and generally responsible for nosocomial infections (Diggle *et al.*, 2020). The antibiotics and disinfectants are decomposed by P. aeruginosa forcing us to think for alternatives (Harper D.R. *et al.*, 2014). Lytic bacteriophages are most effective alternatives to handle resistant and biofilm forming P. aeruginosa (Harper D.R. and Enright M.C. *et al.* 2011). Sewage is known to be rich source of P. aeruginosa and its bacteriophages (Eman M. Marei *et al.*, 2020). In this study, P.aerugonisa was isolated from samples, collected from sewage treatment plant. Eman M. Marei, (2020) isolated P. aeruginosa from Cystic fibrosis patients (Essoh C *et al.*, 2015). Phages will be more in the environment of their corresponding bacteria (Xie *et al.*, 2005). P. aeruginosa phages were also isolated from sewage samples (Essoh C *et al.*, 2015; Chegini *et al.* 2020). P. aeruginosa was found to be multidrug resistant (Pallavali *et al.*, 2017; sonika Sharma *et al.*, 2021; Sharahi JY *et al.*, 2020) and the same is confirmed in the present study. Isolates of P. aeruginosa revealed 50%, 63.9%, 16.7%, 8.3%, and 16.7% resistance to imipenem, cefepime, amikacin, tobramycin, gentamicin, respectively (Hosu MC *et al.*, 2021). Brzozowski *et al.* 2020 reported lower reistanceto imipenem and cefepime and higher resistance to tobramycin, amikacin, and gentamicin. P. aeruginosa lytic phages have been used in several studies to cure infections caused by MDR strains of P. aeruginosa. phage in this study infects and destroys five MDR P. aeruginosa bacterial strains. The lytic phage PAXYB1 isolated from waste water was used to kill P. aeruginosa strain PAO1 and other clinical isolates (Yu X *et al.*, 2017). The lytic phage AZ1 was isolated against the MDR clinical strain of P. aeruginosa-2995 and this phage activity was determined against P. aeruginosa 2995 in both planktonic cells and the biofilm (Jamal M *et al.*, 2017). In another work, bacteriophages were isolated from sewage and tested against MDR-bacterial isolates

(P. aeruginosa, S.aureus, K. pneumoniae and E. coli) from patient with septic wound infections. These phages displayed perfect lytic activity against the MDR bacteria causing septic wounds and concluded that phages are therapeutic options for treating septic wounds (Pallavali RR *et al.*, 2017). P. aeruginosa phage isolated from sewage of Ilam had significant effects against a variety of clinical and general laboratory strains of P. aeruginosa (Azizian R *et al.*, 2015). In present study, 90% of P. aeruginosa cells were inactivated in 6 hours whereas in study of Sonika Sharma *et al.*, (2021), 90% of P. aeruginosa cells inactivated in 7 hours. P. aeruginosa phage burst size is 151 PFU per cell. P. aeruginosa phages with larger burst sizes are phage PAXYB1 (burst size approximately 141 PFU/cell) and phage PPA-ABTNL (burst size approximately 110 PFU/cell) (Yu X *et al.*, 2017, Cao Z *et al.*, 2015), phage ø/PSZ1 (burst size approximately 100 PFU/cell) and phage ø/PSZ2 (burst size approximately 100 PFU/cell) (El Didamony G *et al.*, 2015).

In this study, Phages were PB1 like phage in morphological appearance, having icosahedral head with a diameter of 80nm X 75nm and long non contractile tail of 130nm based on TEM. The phage PPaMa 1/18 had large, icosahedral head with diameter of 90 nm × 75 nm and long contractile tail of 215nm in length and SL2 phages were with head of 120 nm diameter and tail of 170 nm length. (Jurczak-Kurek A *et al.*, 2016). The phage DRL P1 had icosahedral head with diameter of 197.47nm and tail of length 94.54 (Sonika Sharma *et al.*, 2021). PAK-P1 like phages showed morphology of 130 nm tail and a 67–70 nm head (Essoh C et al., 2015). The P. aeruginosa bacteriophage in this study had 80% of host range as it infected four out of five P. aeruginosa strains. PPaMa1/18 phage had the broadest host range with 85.7% infectivity of P. aeruginosa isolates (Majdani R, Shams Ghahfarokhi E., 2022). The cluster of phages which belonged to six genera also had 80% host range as they infected 16 out of 20 P.aureginosa isolates (Essoh C. *et al.*, 2015). The effect of phage treatment on the bacterial biofilms was tested by crystal violet staining as reported by Sonika Sharma *et al.*, (2021), whereas the anti-biofilm activity was tested using safranin staining by P. Gupta *et al.*, (2017). In this study, the biofilms were degraded by 50% due to phage infectivity for 4hrs, where as 55%, 41%, and 33% of biofilms were degraded after 12 h, 24 h, and 48 h of incubation with phage, respectively (Sonika Sharma *et al.*, 2021).

## CONCLUSION

P. aeruginosa is a multi drug resistant, opportunistic pathogen forming biofilms and phage therapy is belived to be only solution. P. aeruginosa isolates and PB1 like phage particles were isolated from sewage samples collected from sewage treatment plant. PB1 like phage was featured with large burst size of 151PFU/cell, host inactivation in 6 hrs, 80% of host range and had icosahedral head with a diameter of 85 x 80nm and long non contractile tail of 135nm. PB1 like phages are suitable for lysis of broad range MDR Pseudomonas biofilms hence can be used in phage therapy.

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# Acinetobacter Baumanii as Cause of Septicemia in Burn

**Naveen Raj S[1], Ravi Kumar Chittoria[2], Amrutha JS[3]**

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**Abstract**

Burn patients are prone to sepsis mainly due to the loss of the skin barrier and immunosuppression. Burn wound infections may originate from the patient's endogenous skin, gastrointestinal, and respiratory flora or may be transferred through contact with contaminated external surfaces and infected hands of healthcare workers. Burn patients are vulnerable to infections, especially infections with multidrug resistant organisms which are usually healthcare associated. Healthcare associated infections refer to infections affecting patients in a hospital or other healthcare facility that were not present or incubating at admission. These include occupational infections among healthcare workers and infections acquired in the hospital or other healthcare facilities but appearing after discharge. This study highlights the role of Acineto bacter baumanii as the cause of septicemia in burns.

**Keywords:** Acineto bacter Baumani; Septicemia; Burns.

## INTRODUCTION

Acinetobacter species are known to cause healthcare associated infections due to their ability to survive for long periods of time on hospital environmental surfaces and their propensity to develop drug resistance.[1-2] Acinetobacter baumannii are Gram-negative, nonmotile, nonglucose fermenters, and catalase positive and oxidase negative coccobacilli that possess virulence factors such as porins, capsular polysaccharides, lipopolysaccharides, phospholipases, outer membrane vesicles, metal acquisition, and protein secretion systems. All these contribute to its pathogenesis.[3] The prevalence rate of Acinetobacter infection varies from region to region. A prevalence rate of 19.2% was reported in Asia, 17.1% in Eastern Europe, and 14.8% in Africa. The lower prevalence rate was observed in Western Europe (5.6%), Oceania (4.4%), and 3.7% in North America.[4] Despite the challenges, it poses only a few studies have been done in Nigeria. A prevalence rate of 14% was reported in the Intensive Care Unit of a tertiary hospital in Southwest Nigeria, while none has been documented in North-western Nigeria.[5]

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Multidrug resistant (MDR) Acinetobacter is an emerging organism isolated from burn patients which poses a challenge to the management of burns wound sepsis and is associated with mortality rates as high as 35%.[6] Acinetobacter species is also an important etiological agent for healthcare associated infections (HCAIs) outbreaks, especially in hospital Intensive Care Unit (ICU) settings.

## MATERIALS AND METHODS

This study was conducted in the Department of Plastic Surgery at a tertiary care center in South India after getting the departmental ethical committee approval. Informed written consent was taken from the patient's parents. A 2-year-old male child had accidental scald burns by skipping and falling into hot boiled water. On examination, there are 2nd degree deep and superficial scald burns involving the chest, abdomen, right arm and elbow, lower limbs, back, buttocks, genitalia, and neck (Total body surface area - 48%) (fig. 1).

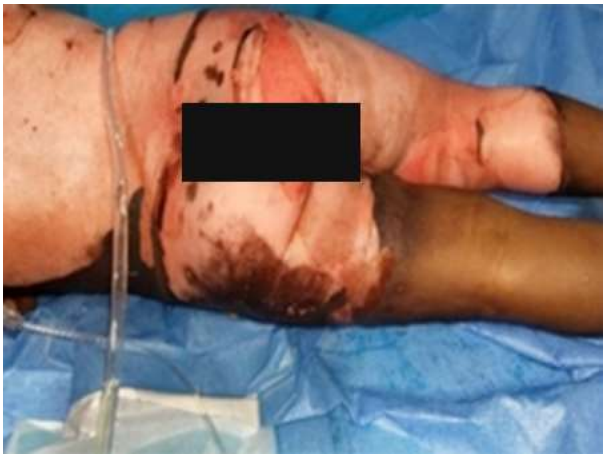

**Fig. 1:** Case of Scalds with septicemia

The childhas been admitted to the Burns ICU. Blood culture taken from the central line shows the growth of Acineto bacter baumanni as cause of septicaemia. Antibiotics and maintenance fluids started according to pediatric orders. The child was managed by Inj. Cloxacillin, Cefoperazone and sulbactam, morphine. In view of persistent fever spikes and worsening respiratory status antibiotics were hiked (Minocycline and Vancomycin). He underwent wound dressing in EMS OT under GA. Paediatric ICU, Critical Care Unit (CCU) consultations are done in view of persistent hypotension. The child was started on Inotropes. The child was intubated by the CCU team in view of respiratory distress. The inotropes dose escalated. The child has one episode of cardiac arrest and was given CPR, calcium, and bicarbonate injections and was revived. He had another apnoeic episode, and despite all resuscitative measures, the Child could not be revived and was declared expired.

## RESULTS

In our study, blood culture grew Acinetobacter Baumannii (fig. 2) & lead to septicemia & death of the patient.

## DISCUSSION

Burn patients are prone to sepsis mainly due to the loss of the skin barrier and immunosuppression. Burn wound infections may originate from the patient's endogenous skin, gastrointestinal, and

Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER)
Dhanvantri Nagar, Puducherry- 605009
Hospital Information Services Unit
Department of PLASTIC SURGERY-Discharge Summary

BLOOD CULTURE REPORT

| | | | |
|---|---|---|---|
| HOSP NO | : J-458408 | SAMPLE NO | : 307202306170 |
| NAME | : [redacted] | ADVISED DATE & TIME | : 20-Feb-23 06:26 |
| AGE/SEX | : 2/M | REPORT DATE & TIME | : 21-Feb-23 09:11 |
| WARD/ROOM/BED | : BURNS ICU 4502/GENERAL/9 | SERVICES | : PLASTIC SURGERY |
| CLINICIAN NAME | [redacted] | SPECIMEN | : BLOOD CULTURE |

INITIAL INFORMATION

| | |
|---|---|
| Diagnosis | 48% burns with persistent spikes |
| No. of bottles received | 1 |
| Source | CENTRAL LINE |
| Blood culture bottle type | BACTALERT PEDIATRIC |

REPORT

Pathogen is grown after aerobic incubation in automated blood culture system.

| | |
|---|---|
| TIME TO POSITIVITY | 8.00 Hours |

Time-to-positivity (TTP) denotes the time taken for the blood culture bottle to flag-positive. TTP of blood cultures is considered to be a predictor of the clinical outcome for bacteremia.Lower TTP values are associated with worse clinical outcome; whereas higher TTP values are associated with better clinical outcome.

DIRECT BOTTLE GRAM STAINING

GRAM NEGATIVE BACILLI

ORGANISMS GROWN IN CULTURE

ACINETOBACTER BAUMANNII

**Fig. 2:** Blood culture report showing growth of Acinetobacter Baumannii

respiratory flora or may be transferred through contact with contaminated external surfaces and infected hands of healthcare workers. Burn patients are vulnerable to infections, especially infections with multidrug resistant organisms which are usually healthcare associated. Healthcare associated infections refer to infections affecting patients in a hospital or other healthcare facility that were not present or incubating at admission. These include occupational infections among healthcare workers and infections acquired in the hospital or other healthcare facilities but appearing after discharge. Acinetobacter baumannii are Gram-negative, nonmotile, nonglucose fermenters, and catalase positive and oxidase negative coccobacilli that possess virulence factors such as porins, capsular polysaccharides, lipopolysaccharides, phospholipases, outer membrane vesicles, metal acquisition, and protein secretion systems. All these contribute to its pathogenesis.[7]

Bacterial transmission occurs commonly through incubators and ventilation devices such as respirators. Human infection can occur through water distribution systems and contaminated surfaces. Constant wound surveillance procedures in the hospital help in identifying the risk of infection.[8]

The common infection sites include major infected burns, urinary tract, respiratory, and brain

infections including meningitis. The organism is multidrug resistant there by increasing the morbidity and mortality in burn patients.[9]

## CONCLUSION

Acinetobacter Baumanii causes septicemia in burns and is intrinsically resistant to most antibiotics causing significant morbidity and mortality in burns patients. Large randomized studies are required to substantiate the results of our study.

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*Original Article*

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# Role of Low Level Laser Therapy in Adult Burn Patient

**Kishore Kumar[1], Ravi Kumar Chittoria[2], Amrutha JS[3]**

**How to cite this article:**
Kishore Kumar, Ravi Kumar Chittoria, Amrutha JS/Role of Low level laser therapy in Adult Burn Patient/J Microbiol Relat Res. 2023;9(1): 25–27.

**Abstract**

Low level laser therapy helps in various aspects of wound healing. It has effect on cell proliferation, metabolism, angiogenesis, apoptosis and inflammation. This study assess the role of Low level laser therapy (*Low Level Laser Therapy*) in adult burns patients

**Keywords:** Low Level Laser Therapy; Adult Burns; Wound Healing.

## INTRODUCTION

Burns injury is one of the important factors contributing to mortality in a developing country like India. Aim of this case report is to assess the role of Low level laser therapy in healing of burn wounds in adult burns patients. Clinical examination of the wound site before and after the use of Low level laser therapy was done. The normal pace of wound healing and epithelialization is at the rate of Imm/day. Optimum recovery requires the wound bed and the patient to be fit. The advanced wound healing therapies aim to hasten the process of wound healing by expediting the advancement of epithelial edge of the wound. Many newer techniques have been used to advance the epithelialization such ad *Low Level Laser Therapy*.

**Authors Affiliation:** [1]Junior Resident, Department of General Surgery, [2]Professor, [3]Senior Resident, Department of Plastic Surgery, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry 605006, India.

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**E-mail:** drchittoria@yahoo.com

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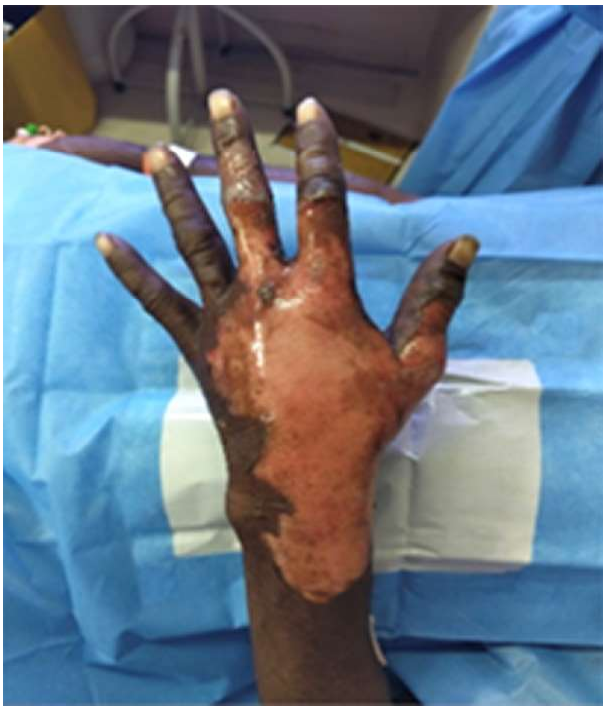
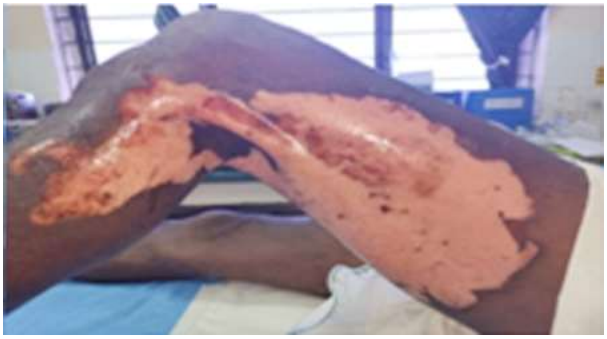
## MATERIALS AND METHODS

The study is done in a tertiary care hospital in South India. The subject is a 55 year old male patient, known hypertensive for 3 years, Alleged history of accidental thermal burn with fire flame while saving his wife from burn. Patient sustained 2nd degree Flame Burn 15% TBSA involving Left Hand, Left Thigh and Leg back and Right Leg. Admitted in Burns ICU, managed with antibiotics, IV Fluids, analgesics. Six sessions of Low level laser therapy following which the wound improved well (Fig. 3).





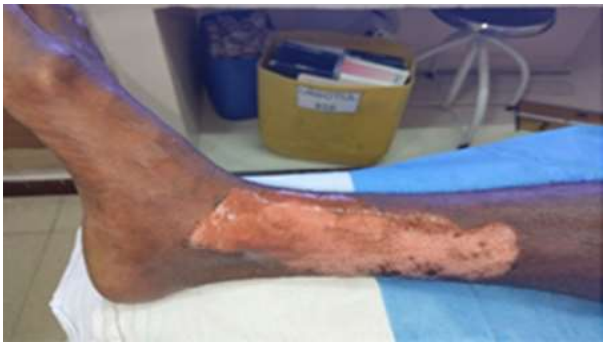
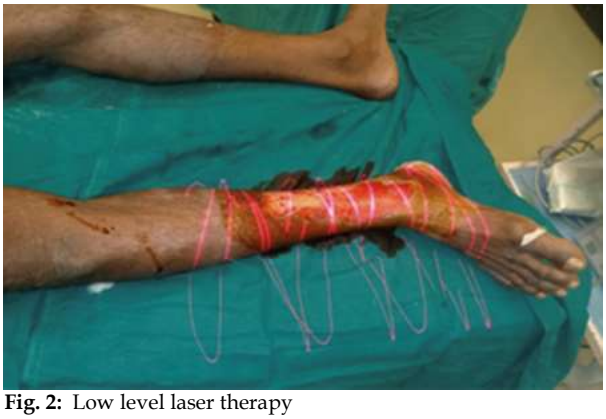


**Fig. 1:** 15% second degree burns at the time of admission



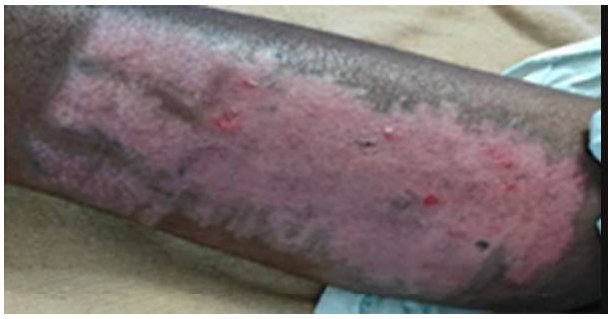
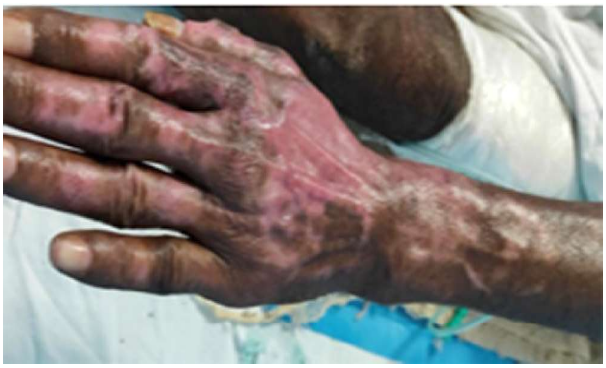
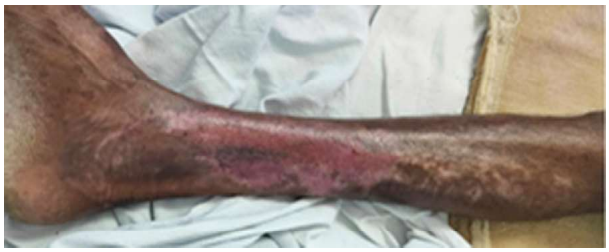


**Fig. 2:** Low level laser therapy

## RESULTS

*Low Level Laser Therapy* is useful in improving the wound healing of burns in adult patients.





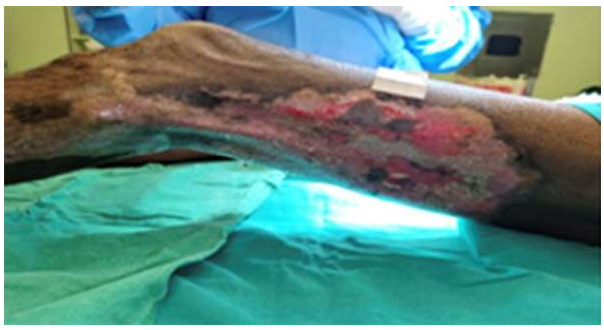


**Fig 3:** after 6 session of Low level laser therapy

## DISCUSSION

Low level laser therapy is generated from G-As (gallium-arsenide) laser. *Low Level Laser Therapy* acts by photo biomodulation. It has effect on cell proliferation, metabolism, angiogenesis, apoptosis and inflammation. Effective *Low Level Laser Therapy* utilises wavelength of red to near infrared (600-1070 nm). *Low Level Laser Therapy* acts on cytochrome c oxidase, promotes nuclear factor kappa b which promotes cell proliferation and anti-apoptotic action. It also upregulates VEGF which promotes angiogenesis. Low level laser is applied by scanning mode and adjusted to cover the region of the wound. Application is for 5-10 minutes per weekly session. It has a stimulatory effect on raw areas and wounds by improving granulation. It softens scars by reducing fibrous tissue formation, improves blood supply and promotes nerve regeneration. It has an anti-inflammatory action, the mechanism of which is not clearly elucidated.

## CONCLUSION

Low level laser therapy is found to be useful in promoting wound healing in adult burns patients.

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[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

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[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

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[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

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[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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