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# Study of antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from burn patients swab

**Sathe Shahadeo Narayan, RM Gulve**
Department of Microbiology, Mrs. K. S. K. Arts, Science and Commerce College, Beed. Maharashtra, India

**Abstract**
The present study performed on patients admitted in burns unit to determine *Pseudomonas aeruginosa* of burn wound infection and to study their antibiogram. It is an opportunistic pathogen that develops life threatening infection in patients with immunological system defect like burn patient.
In this study during the period of last six month [1June to 31December2018] total samples of 135 burn patients swab was collected using sterile cotton swabs from hospitalized in the Kasturba Hospital, Mahalaxmi, Mumbai. *Pseudomonas aeruginosa* was identified by standard bacteriological method. The antibiotic resistance patterns using different antimicrobial agents like [Ceftazidime, Cefepime, Colistin, Gentamicin, Amikacin, Ciprofloxacin, Imipenem and Levofloxacin] were performed for all the isolates using Kirby Bauer's disc diffusion method.
*Pseudomonas aeruginosa* were isolated from 135 clinical samples and all these isolates multidrug resistance *Pseudomonas aeruginosa*. The resistance rate to various antibiotics were as Ceftazidime [26.66%] Cefepime [8.88%] Colistin [14.81%] Gentamicin [34.07%] Amikacin [13.33%] Ciprofloxacin [32.59%] Imipenem [16.80%] and Levofloxacin [15.83%]
This hospital-based study will be help to implement better infection control strategies and improve the knowledge of antibiotic resistance patterns among clinicians. The finding of study also helpful for identifying the common bacteria causing burn wound infection. To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies in our country.

**Keywords:** antibiotic resistance pattern, antibiotics, burn wound infection, multidrug resistance (MDR), *Pseudomonas aeruginosa*

## Introduction

Burn injury is a major significance problem in the world. It has been estimated that 75 % of all deaths following burns are related to infection. It concluded that the burns patients were most commonly infected with the *Pseudomonas aeruginosa* gram-negative bacteria. Burns are one of the most common and devastating forms of trauma and a major public health concern in all around the world. Globally an estimated 75% death occurs annually. And most importance to do not require hospitalized in longer time. The emergence worldwide of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections (Virendra S Kolhe1, Antibiotic Resistance Pattern in Aerobic Gram Negative Bacterial Infection in Burn Patient's at Tertiary Care Hospital in Maharashtra, May 2017). Antibiotics first introduced were considered as a miraculous drug. Unfortunately, most of the cheaper antibiotics lost their efficacy due to emergence of resistance among bacteria. Expensive and complicated antibiotics were introduced to tackle simple infections. *Pseudomonas aeruginosa* is an aerobic, nonfermenting, Gram-negative bacillus. It is commonly involved in opportunistic nosocomial infections. *Pseudomonas aeruginosa* develops resistance against maximum all antibiotics by several mechanisms like, multi-drug resistance like that aminoglycoside modifying enzymes and mutations in different chromosomal genes (Abdul Samad1, 2017) [1]. Pseudomonas aeruginosa is an aerobic, motile, gram negative rod. It belongs to the family of pseudomonadaceae. *Pseudomonas aeruginosa* being an opportunistic human pathogen, it is the fatly cause of nosocomial infections, generally patients are admitted to intensive care units (ICU). It can become resistant through mutations in the chromosomal genes which regulate the resistance genes (Mohanasoundaram, 2011 Jun) [7]. It has been found by many messengers that the distribution of various species of bacteria from burn wound surfaces. Burn patients are ideal hosts for opportunistic infections. The burn site remains relatively sterile during the first 24 hour; thereafter, colonization of the wound by gram negative bacteria (Latika Sharma, 08 January 2017) [5]. Now a day, the most widespread methods used for microbial monitoring of burn wounds are swab culture and biopsy culture. The swab culture is a non-invasive and less expensive method, (Mohammad Ali Bahar, August 2008.). Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality (AL-Aali, 2016) [2]. *Pseudomonas aeruginosa* is most challenging organisms involved in a multiplicity of infections. It is highly leading cause of nosocomial infections and is associated with a high mortality rate. Regional variations in antibiotic resistance patterns for different organisms including *Pseudomonas aeruginosa* also occur, which could be due to differences in antibiotic prescribing practices (Mubashir A. Khan, 2016) [8]. With advancements in burn care over the last 50 years, infection is now the leading cause of death after extensive burn injuries.

Multiple studies over the last decade have shown that 42%–65% of deaths in burn victims are attributable to infection (Anne M. Lachiewicz, 15 December 2020) [3]. The 100% of the isolates were resistant to Tobramycin and 94.10% were resistance to Cefoperazone and Meropenem followed by Amikacin a Gentamicin 47.10% whereas the organisms was resistant to Ciprofloxacin 35.30%. And they noted the *Pseudomonas aeruginosa* was Multidrug resistance (MDRPa) (S Upadhaya*1, October 2015.) [11] This organism is highly pathogenic due to its ability to produce a variety of toxins and proteases. Genus Pseudomonas consists of more than 140 species including *Pseudomonas aeruginosa* primary human pathogen in genus Pseudomonas. The strains are regularly sensitive to cephalosporins, carbenicillin, colistin, gentamicin, polymyxin, quinolones, and streptomycin; but a degree of cross-resistance among these agents has been reported. There to public health globally due to the limited treatment options (Rugira Trojan, d19September2016) [10]. Injuries and deaths due to burn are a global concern with regards to public health, accounting for an estimated 265,000 deaths annually. The majority of these occur in low- and middle-income countries and almost half occur in the WHO South-East Asia Region. Approximately, 75% of deaths occur primarily. But India, in the year 2013, a total of 22,177 (5.9%) deaths and 2391 injuries occurred as a result of some form of accidents due to fire. Fire accidents constituted 5.5% of the un-natural causes of accidental deaths, of which 65.7% of those killed were females who out-numbered males (34.3%).The most common causes of work-related burns are contacted with chemicals or hot liquids, followed by electricity and then molten or hot metals. The organisms responsible for infections in patients with severe burns may be endogenous or exogenous and include bacteria, fungus, and viruses which can change over time in the individual patient (Sayoni Datta1, August 2016) [12]. In the recent years, *Pseudomonas aeruginosa* has over taken *Staphylococcus aureus* in causing wound infections. In the past three years, some of the Indian studies shown *Pseudomonas aeruginosa* as the most predominant isolate from wound infections (Babu, 2018) [4]. The organisms are mainly derived from the patient's gastro intestinal and upper respiratory tracts as well as from the hospital environment (T. Sabetha1, Study on Bacterial Flora of Burn Wound Infection: A Need for Microbiological Surveillance in Burn Units, Number 2017). *Pseudomonas aeruginosa* is one of the leading causes of hospital-acquired as well as community-acquired Infections. *Pseudomonas aeruginosa* continues cause complications in hospital-acquired infections. Penicillin when first discovered and used for the treatment was a magic drug. A single injection of penicillin could cure a life-threatening infection. Unfortunately, with time due to malpractices of natural causes, most of the cheaper antibiotics have lost their efficacy, and more expensive and complicated antibiotics were introduced and marketed to combat simple infection (Vikas Chandra Yadav1,: October 17, 2016) [16]. Methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci, extended spectrum beta- lactamases (ESBL) and metallo betalactamases (MBL) producing Gram-negative bacteria have been emerging as serious challenges in hospitalized patients. These organisms can be transmitted easily from one patient to another. Thus, the burn units are common places where explosive and prolonged outbreaks of infections caused by resistant organisms occur (Vimal S. Rathod1, March 2017). Burn injury is a major problem in many parts of the world. The emergence worldwide of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections (Virendra S Kolhe1, Antibiotic Resistance Pattern in Aerobic Gram Negative Bacterial Infection in Burn Patient's at Tertiary Care Hospital in Maharashtra, May 2017). It has been implicated in various nosocomial infections e.g., pneumonia, urinary tract infection, skin and soft tissue infections, in severe burns and wound infections. *Pseudomonas aeruginosa* is relatively resistant to many antibiotics. The rate of antibiotic resistance *Pseudomonas aeruginosa* is increasing in many parts of the world, in particular to β-lactams, aminoglycosides, and fluoroquinolones. Its general resistance is due to a combination of factors. It is resistant to antimicrobial agents, due to low permeability of its cell wall. It has the genetic capacity to express a wide range of resistance mechanisms. It can become resistant through mutations in the chromosomal genes which regulate the resistance genes (Yasser Mahmoud Alkeshan 1, Apr. 2016) [20]. The rapid increase of drug resistance in clinical isolates of this opportunistic pathogen has been of worldwide concerns. It is also the most common gram-negative bacteria found in nosocomial infections. Many *Pseudomonas aeruginosa* infections occur after patients have been hospitalized. Several factors that account for the success of *Pseudomonas aeruginosa* are as follows it can utilize a wide range of nutrients (Zeynab Golshania*, Oct 2012) [21].

The present study was undertaken in the Department of Microbiology. In order to determine aerobic bacterial isolates from burn wound swabs in hospital setting and describe their resistance patterns. The aim of study characterizes the pathogenic bacteria from pus samples and to determine their antibiotic susceptibilities. Objective of this study were to find out resistance pattern of *Pseudomonas aeruginosa* from burn patient swabs.

**Materials and Methods**

From a total 135 swabs was collected and isolates from burn wound. *Pseudomonas aeruginosa* it used Pseudomonas isolation agar base, glycerol for isolation of selective species. The culture was prepared for bacterial isolated and pours on Muller-Hinton agar plate it used different antibiotics like that Colistin, Doxycycline Hydrochloride, Tobramycin, Azithromycin, Amikacin, Meropenem, Cefoperazone/ Sulbactam, Gentamicin, Ciprofloxacin, and Ceftrixone all these made from Himedia Laboratories Mumbai, India. The numerical data obtained from the study were analysed. The obtained data was expressed in percentage of male and female respectively. This study is the last six-month hospitalized burns patients collected the pus swab sample. Isolate, inoculate and performed the antibiotic resistance by using different antibiotics as for the guidelines of clinical and laboratory standard institute and interpret. The total numbers 135 *Pseudomonas aeruginosa* species are isolates from burns patients' swabs in the duration of 01 June 2018 to 31 December 2018. The burn patient swabs are collected and

done the work in the Microbiology Laboratory. Pus swabs are collected and inoculated on the MacConkey's agar plates as well as selective Pseudomonas agar base media make by Himedia. Bacterial isolations were identified predictable methods. Antibiotic resistance tests were performed using Kirby Bauer's disc diffusion method as per clinical and Laboratory Standard Institute (CLSI) guidelines. The current study was undertaken in the Department of Microbiology Mrs. K. S. K. Arts, Science and Commerce College, Beed. Petri dish containing 20 to 25 ml of Mueller-Hestonagar plates pour with a 24 hours old broth culture of the microbial strains and apply the antibiotic disc on to the seeded plates. After overnight incubation at 37'C the zone of inhibition around the disc was show and related with the standard strains (ATCC *Pseudomonas aeruginosa* 27853) as sanctioned by the CLSI manual. Zone of inhibition was noted in millimetre scale. And the result noted on the basis of the zone inhibition size as related with the standard strain were interpreted resistant.

## Result

The highest resistance was recorded the Gentamicin which was 34.07% out of 135 number isolates of *Pseudomonas aeruginosa* and lowest resistance was the noted that the Cefepime 8.88%out of 130 number isolates of *Pseudomonas aeruginosa* and maximum resistance was noted that the Genatmicin (34.07%) followed by Ciprofloxacin (32.59%), Ceftazidime (26.66%), Imipenem (16.80%), Levofloxacin (15.83%), Colistin (14.81%), Amicacin (13.33%), Cefepime (8.88%) it show in details in Table no. 01.

In the Table no. 02 show the details about age group and percentage of pus swabs. And in Table no. 03 show the percentage of burns patients male and female.

**Table 1:** Antimicrobial resistance rate of the *Pseudomonas aeruginosa.*

| The antibiotic tested | *Pseudomonas aeruginosa* (no.135) | | |
|---|---|---|---|
| | No. of the isolates tested | Number of the resistance isolates | |
| | | Number | Percentage (%) |
| Ceftazidime | 135 | 36 | 26.66% |
| Cefepime | 130 | 12 | 8.88% |
| Colistin | 133 | 20 | 14.81% |
| Gentamicin | 135 | 46 | 34.07% |
| Amikacin | 135 | 18 | 13.33% |
| Ciprofloxacin | 135 | 44 | 32.59% |
| Imipenem | 125 | 21 | 16.80% |
| Levofloxacin | 120 | 19 | 15.83% |

**Table 2:** Distribution of *Pseudomonas aeruginosa* isolates from pus swabs 135 according to age group and percentage.

| Specimens | | Group of age | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Burns Patients pus swabs | | 0 - 15 | | 16 - 30 | | 31 - 45 | | 46 - 60 | | 61 - above | |
| | | 37 | | 40 | | 30 | | 15 | | 13 | |
| % of pus swabs | | 27.41 | | 29.63 | | 22.22 | | 11.11 | | 9.30 | |
| Sex | | M | F | M | F | M | F | M | F | M | F |
| Male | Female | 13 | 24 | 14 | 26 | 13 | 17 | 10 | 5 | 7 | 6 |
| % M | % F | 35 | 65 | 35 | 36 | 43 | 57 | 67 | 33 | 54 | 46 |

**Table 3:** Percentage of burns patients Male and Female.

| Burns Patients | Total | Male | Female | Male % | Female % |
|---|---|---|---|---|---|
| | 135 | 57 | 78 | 42.22 | 57.78 |

## Discussion

*Pseudomonas aeruginosa* is a gram negative aerobic rod belonging to the bacterial family pseudomonadaceae. The resistance of Pseudomonas species to antibiotics has amplified considerably over the past few years and therefore need to be assessed regularly to have a clear opinion of clinical outcome of different therapeutic options. (Abdul Samad1, 2017) [1] The burn wound infection is one of the frequent and severe complications in patients who have sustained burns. In the Indian study the mortality rate of the burned patients was reported to be 38%. The high rates of resistance to Aminoglycosides antibiotics, including Tobramycin (82%), Amikacin (73%), and Gentamicin (80%). However, increasing Imipenem resistant strains have also been reported. This can be attributed to females being more exposed to household fire while cooking and also suicidal and dowry deaths. This finding of female predominance in burn patients correlated with other studies done in different hospitals in India.(Sayoni Datta1, August 2016) [12] The resistance pattern in *Pseudomonas aeruginosa* showed Gentamicin: 51%, Amikacin: 24% and Fluoroquinolone (58%) groups with a statistically significant ($p<0.05$) rise in resistance to Ciprofloxacin. They are distributed the patients according to age groups like that 0 – 15, (37) 16 – 30, (40) 31 – 45, (30) 46 – 60, (15) 61 – above. The number of Male and Female patients noted that the male 56 and female 79. We were tested for antibiotic resistance pattern by using disk diffusion method as per Clinical and Laboratory Standards Institutes guideline. Maximum antibiotic resistance was noted that the Gentamicin (34.07%) followed by Ciprofloxacin (32.59%), Ceftazidime (26.66%), Imipenem (16.80%), Levofloxacin (15.83%), Colistin (14.81%), Amikacin (13.33%), Cefepime (8.88%). Burn injury is the major significance public health problem worldwide. On the basis number of studies have been conducted that the various locations in India and in the outside of countries. To the determine burns wound infections and consistent of the antimicrobial resistance patterns. *Pseudomonas aeruginosa* isolates 90 to 95% were resistance to Gentamicin, Amikacin and Ciprofloxacin. The 65% of isolates were resistance to Ceftazidime and 23% of isolates resistance to Meropenem. And the *Pseudomonas aeruginosa* 72% of isolates were sensitive to Piperacilli/Tazobactam and 55.8% of isolates were sensitive to Meropenem. It was obtained from burns wound infection found tube multidrug resistant same as on our study 87.41% isolates are multidrug resistance and it was correlated to these studies. The *Pseudomonas aeruginosa* isolates of 41.425 were resistant to Piprracillin, 85.18% of isolates were resistant to Amikasin, 89.22% of isolates were resistant to Gentamicin, 78.81% of isolates were resistant to Ciprofloxacin, 4.54% of isolates were resistant to Impenem and 21.8% of isolates were resistance to Ceftazidimine. The 100% of the isolates were resistant to Tobramycin and 94.10% were resistance to Cefoperazone and Meropenem followed by Amikacin and Gentamicin 47.10% whereas the organisms were resistant to

Ciprofloxacin 35.30%. And they noted the *Pseudomonas aeruginosa* Multidrug resistance (MDRPa). (S Upadhaya*1, October 2015.) [11] The *Pseudomonas aeruginosa* was common isolates and it show resistance of 75%. Commonly used of 9.80% of *Pseudomonas aeruginosa*, isolates ware as the resistance to the entire three drugs like that Piperacillin/ tazobactam, Ceftazidimer and Gentamicin whereas the multidrug resistant. And other side it was noted that the 100% sensitive to Imipenem. (Vimal S. Rathod1, March 2017) The isolates of *Pseudomonas aeruginosa* noted that the 22.30% of Ticarcillin was resistance. But the high resistance rate was found in meropenem and ticarcillin in the all hospital. The isolates of *Pseudomonas aeruginosa* 21.2%, in the gentamicin and ciprofloxacin are resistance was 56.8% and 61.3% respectively. The Ceftazidime, Amikacin, Netilmicin, Piperacillin, Meropenem and Piperacillin/tazobactam are resistance to 57%, 56.80%, 61.30%, 50%, 45.40% and 45.40% respectively noted. Out of 204 *Pseudomonas aeruginosa* isolations maximum sensitive Imipenem (81.3%) and the followed by sensitive Piperacillin/tazobactam, Meropenem and Piperacillin are (76.4%), (70.5%) and (67.6%) respectively. The age group of in our study maximum burns patients was the 16 – 30. It was difference to study the other research scholars. In this study noted that the more incidence was happen in female then the male as on seen 57.78% female and 42.22% male are burns. Most of the burn injuries were happening by thermal. According to our knowledge and studies the thermal burns are the maximum common type of burns injury studied (Vimal S. Rathod1, March 2017). It study the maximum resistance 34.07% found in Gentamicin and minimum resistance was noted that the Cefepime 8.88%. It was co-related to the studied by (Sayoni Datta1, August 2016) [12]. In our study the age group 30 – 40 affected on the mostly burns due to lack of illutricity and fire it was correlated to studied (Sayoni Datta1, August 2016) [12]. In this study most of the 135 isolates of Ceftazidime and Cefepime was noted the26.66% and 8.88% respectively resistance. It was correlated to the studied by (T. Sabetha1, Study on Bacterial Flora of Burn Wound Infection: A Need for Microbiological Surveillance in Burn Units, Number 2017). This studied the number of resistance isolates and percentage resistance which are shown in the tabl e No. 1. It was some correlated to the studied by (Virendra S Kolhe1, Antibiotic Resistance Pattern in Aerobic Gram Negative Bacterial Infection in Burn Patient's at Tertiary Care Hospital in Maharashtra, May 2017). The antibiotic resistance pattern performed by Kirby Bauer's disc diffusion method and to use the different drugs which are commonly used. In this study all these noted results are show in the table No. 01(S Upadhaya*1, October 2015.) [11] The *Pseudomonas aeruginosa* was common isolates and it show resistance of 75%. Commonly used of 9.80% of *Pseudomonas aeruginosa*, isolates ware as the resistance to the entire three drugs like that Piperacillin/ tazobactam, Ceftazidimer and Gentamicin whereas the Multi drug resistant. And other side it was noted that the 100% sensitive to Imipenem. (Vimal S. Rathod1, March 2017). The isolates of *Pseudomonas aeruginosa* noted that the 22.30% of Ticarcillin was resistance and it was lass compared Makkah (56.30%). Highly resistance to tricarcillin (93%) was noted in the study from Turkey. In this study the *Pseudomonas aeruginosa* resistance to piperacillin 17.30% and compared to other published studied was Riyadh (54%) resistance to piperacillin. Overall the study compared to other hospitals data drug resistance are different found.

## Conclusion

From this present study, we conclude that the pseudomonal infection was the most commonly occur in generally burn patient. Among the Cefepime, Amikacin and Colistin demonstrated minimum resistance against the *Pseudomonas aeruginosa.* Therefore, the use of agents should be restricted to serve nosocomial infections, in order to avoid rapid emergence of resistant strains. This type of study should be carry continue since six month and to detect the resistance pattern.

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