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### Key Words

Nosocomial wound infections, pseudomonas aeruginosa, antimicrobial susceptibility testing

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**Received:** 24 January 2024

**Accepted:** 28 February 2024

**Published:** 26 March 2024

**Citation:** Milind Davane, Sanjivani Mundhe, Basavraj Nagoba and Asha Pichare, 2024. Nosocomial Wound Infections with Special Reference to Pseudomonas Aeruginosa. Res. J. Med. Sci., 18: 583-588, doi: 10.36478/makrjms.2024.5.583.588

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## Nosocomial Wound Infections with Special Reference to Pseudomonas Aeruginosa

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### ABSTRACT

Nosocomial wound infections are additional afflictions to patients which are associated with increased morbidity, mortality, increased hospital stay and treatment cost. P. aeruginosa is widespread in natural environments and an opportunistic pathogen causing variety of nosocomial infections including wound infections. It is found to be intrinsically resistant to many classes of antimicrobial agents. Two hundred specimens of pus were collected from patients with nosocomial wound infections. The specimens of pus were immediately inoculated on Blood agar and MacConkey's agar. The inoculated plates were incubated at 37°C for 24 hours. The colonies showing typical morphology of P. aeruginosa on Blood agar and MacConkey's agar, are identified by using biochemical reactions were processed further for antimicrobial susceptibility testing. The most common pathogen isolated was Staph. aureus 71 (30.87%) followed by P. aeruginosa 63 (27.39%), Klebsiella spp. 31 (13.48%), Proteus spp. 21 (09.13%), E. coli 17 (07.39%), Citrobacter spp. 13 (05.65%), Acinetobacter spp. 9 (03.91%) and Strep. pyogenes 5 (02.17%). Among the antimicrobial agents used, the most effective antimicrobial agent found was imipenem (98.41%), followed by amikacin (65.07%), meropenem (57.14%), cefoperazone (53.96%), gatifloxacin (49.20%), ceftazidime (46.03%), ciprofloxacin (44.44%), gentamicin (39.68%) and piperacillin (12.69%) Nosocomial wound infections caused by P. aeruginosa continues to be an important problem to be thought seriously. Improper, unnecessary and indiscriminate use of antimicrobial agents encourage development of resistance and should be avoided. P. aeruginosa isolated from nosocomial wound infections, which are resistant to multiple antibiotics should be paid special attention.

## INTRODUCTION

Nosocomial infections are hospital acquired infections which are not present at the time of admission or in incubation period and are acquired after hospitalization. Nosocomial infections are additional affliction to the patients who are admitted to the hospital for some other serious illness. Microbes are widely distributed in the hospital environment and can reach the sick patients by various routes such as air, water, food, personnel, equipments, linen, catheters, scopes, ventilators and even through the disinfectants, which are used to control microbes. These microbes prevalent in the hospital environment select the patients who have lowered resistance and cause infections, which are difficult to treat. Nosocomial infections are major problems in hospitalized patients, especially in those who are debilitated. These infections are always associated with increased morbidity and mortality, increased hospital stay and treatment cost. The common types of nosocomial infections are wound infections, urinary tract infections, respiratory tract infections, bacteraemia, septicaemia, etc. The microbiology of nosocomial infections is not constant due to several changes in medical and surgical practices such as wide spread use of antimicrobial agents, increased use of invasive diagnostic and therapeutic procedures, and increased use of immunosuppressive agents.

*Pseudomonas aeruginosa* is an important cause of nosocomial infections, especially in patients with lower body resistance and in patients treated with corticosteroids and other immunosuppressive drugs. *P. aeruginosa* is widespread in natural environments and an opportunistic pathogen causing variety of nosocomial infections including wound, urinary, respiratory and septicemia<sup>[1]</sup>. It is found to be intrinsically resistant to many classes of antimicrobial agents<sup>[2]</sup>. Infections due to *Pseudomonas aeruginosa* are seldom encountered in healthy adults but in last four decades, it has become increasingly recognized as an important etiological agent in a variety of serious infections in hospitalized patients with impaired immune defenses and it is currently recognized as one of the leading causes of severe hospital associated infections. *Pseudomonas aeruginosa* is a notorious pathogen causing burns infections and nosocomial wound infections due to its innate resistance to various antimicrobial agents and disinfectants, ability to establish itself widely on moist places in hospital environment, and ability to survive and multiply with minimum nutrients, if moisture is available. Because of its ability to survive in hospital environment and medicaments, it creates threat to patient care. Therefore, continuous and careful monitoring of these objects and sites is necessary to control infections in hospitalized patients. In recent years, it appears that the incidence of nosocomial infections associated with this organism is increasing. It is now a well accepted

fact that there has been an alarming increase in the infections caused by *P. aeruginosa*. In the recent decades, it has assumed an increasingly prominent role as the etiological agent in a variety of serious infections in hospitalized patients. At particular risks are patients who have suffered from major trauma or burns and are exposed to intensive care units. Since the introduction of chemotherapeutic agents and antibiotics, its incidence in hospital acquired infections has been steadily rising and its incidence equals or in some hospitals surpasses in this respect that of *Staphylococcus aureus*.

*Pseudomonas* infection has always been a problem to the clinicians. Conventional therapies with aminoglycosides or antipseudomonal penicillins have their own limitations. The commonest and vexing problem in the tropical climate is contamination by *P. aeruginosa*. Growing bacterial resistance seriously hampers the therapy of infections. The incidence of such multiple drug resistant isolates remains very high in burn units. Wound infection is a common reason for poor wound healing, especially in chronic wounds. *Pseudomonas aeruginosa* is one of the most important causes of nosocomial wound infections and is very difficult to eliminate from infection site. Wound infections by *P. aeruginosa* need special attention, if uncontrolled, may become life threatening. Regular practices of environmental survey and suitable control measures such as the knowledge regarding the site and source of infection, high risk factors along with regular patient oriented surveillance, restricted use and daily change of disinfectants, isolation of infected patients, aseptic and antiseptic procedures and controlled antibiotic therapy can reduce the rates of hospital acquired infections considerably. Various effective measures can help in keeping the rates of hospital acquired infections low. Taking into consideration, a variety of wound infection in hospitalized patients and its ability to develop resistance to various antipseudomonal agents, this study was undertaken.

## MATERIALS AND METHODS

Two hundred specimens of pus were collected from patients with nosocomial wound infections. While selecting the cases for this study, following points were taken into consideration before collecting a specimen of pus:

- Patients developing infections following surgery
- Patients with burns
- Patients developing bed sores following hospitalization for long periods and
- Patients who were not responding to routine antibiotic therapy

Pus swabs from nosocomial wound infection cases were collected in a sterile container by taking all aseptic precautions and transported immediately to

Microbiology Laboratory for further processing. A total of 200 patients with different types of wounds were collected (Table 1).

**Inoculation and Incubation:** The specimens of pus were immediately inoculated on Blood agar and MacConkey's agar. The inoculated plates were incubated at 37°C for 24 hours.

**Identification:** The blood agar and MacConkey's agar after overnight incubation were examined for bacterial growth. The morphology of each different type of colony was noted and each colony was studied for Gram reaction and colony morphology. Each colony was processed further for identification. For this, the colony of Gram positive bacteria was inoculated in glucose broth and that of Gram negative bacteria in peptone water and incubated at 37°C for four hours. The glucose broth culture or peptone water culture of each representative colony was used for studying sugar fermentation reactions (fermentation of glucose, lactose, sucrose mannitol and fermentation of mannitol in Gram positive cocci) and other biochemical reactions such as indole production, methyl red test, Voges-Proskauer test, citrate utilization, urease production, hydrogen sulfide production, catalase and oxidase test. The results of biochemical reactions were recorded in detail, in each case. The isolate was then identified considering its Gram reaction, morphology, colony characters and biochemical reactions using the standard procedures<sup>[3]</sup>. The colonies showing typical morphology of *P. aeruginosa* on Blood agar and MacConkey's agar, are identified by using biochemical reactions were processed further for antimicrobial susceptibility testing.

**Antimicrobial Susceptibility Testing:** The antimicrobial susceptibility pattern of each isolate of *P. aeruginosa* was studied by Kirby-Bauer disc diffusion method<sup>[4]</sup>, using piperacillin (100 µg), ceftazidime (30 µg), cefoperazone (75 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (30 µg) and gatifloxacin (5 µg). The antibiotic discs procured from Himedia Laboratories Ltd, Mumbai were used. For this, the colony of *P. aeruginosa* was inoculated into peptone water and incubated at 37°C for four hours. After incubation, to obtain a standard inoculum size, it was matched with Mc Farland standard 0.5. In each case of less turbidity, tubes were further incubated till desired turbidity was obtained and in case of more turbid broth, plain peptone water was added to make it to match with Mc Farland standard 0.5. After getting desired turbidity, the broth culture was swabbed on Mueller-Hinton agar plate and antibiotic discs were applied. The plates were kept in refrigerator for 5-10 min for diffusion and

then incubated overnight at 37°C and results were noted next morning. The organism was reported susceptible or resistant based on zone diameters compared with standard zone diameters of *P. aeruginosa* from Himedia reference chart. All sterile precautions were taken during the entire procedure<sup>[4]</sup>.

## RESULTS AND DISCUSSIONS

Isolation of *P. aeruginosa* From Nosocomial Wound Infections of the 200 cases of nosocomial wound infections studied, 192 (96%) cases were found positive and eight (4%) cases were found negative. The positivity rate of infection was higher in females (97%) as compared to males (95%). The infection was monobacterial in 158 (82.30%) and polybacterial in 34 (17.70%) cases with two isolates in 30 cases and three isolates in four cases. (Table 2) shows the distribution of pathogens from nosocomial wound infections. The most common pathogen isolated was *Staph. aureus* 71 (30.87%) followed by *P. aeruginosa* 63 (27.39%), *Klebsiella* spp. 31 (13.48%), *Proteus* spp. 21 (09.13%), *E. coli* 17 (07.39%), *Citrobacter* spp. 13 (05.65%), *Acinetobacter* spp. 9 (03.91%) and *Strep. pyogenes* 5 (02.17%). Antibiogram of *P. aeruginosa* Isolated From Nosocomial Wound Infections (Table 3). shows the antibiogram of *P. aeruginosa* isolated from nosocomial wound infections. Among the antimicrobial agents used, the most effective antimicrobial agent found was imipenem (98.41%), followed by amikacin (65.07%), meropenem (57.14%), cefoperazone (53.96%), gatifloxacin (49.20%), ceftazidime (46.03%), ciprofloxacin (44.44%), gentamicin (39.68%) and piperacillin (12.69%).

Of the 200 cases of nosocomial wound infections, 96% cases were found positive. The infection was monobacterial in 82.30% cases and polybacterial in 17.70% cases. Various workers have studied the bacteriology of nosocomial wound infections and reported prevalence and type of monobacterial and polybacterial isolations<sup>[5,10]</sup>. Taiwo *et al.* reported a positivity rate of 83.5%. The monobacterial isolation was seen in 61.5% and polybacterial isolation in 38.7%. However, Lilani *et al.* reported an infection rate of 8.95%<sup>[6]</sup>. In a study carried out on burn wound infections by Mehta *et al.* 97.1% samples were found positive<sup>[7]</sup>. However, Shriyan *et al.* reported a positivity rate of 75% in post operative wound infections<sup>[8]</sup>. Al-Habib *et al.* found a positivity rate of 66.7%<sup>[11]</sup>. However, Garba *et al.* reported an infection rate of 94%<sup>[10]</sup>. Behesthi and Zia in their study on burn wound patients found monobacterial isolation in 73.3% and polybacterial isolation in 26.8% cases<sup>[9]</sup>. The prevalence rate of 96% in the present study is more or less similar to Mehta *et al.*<sup>[8]</sup>. However, it appears to be comparatively more than Taiwo *et al.*<sup>[5]</sup>. Lilani *et al.*<sup>[6]</sup>, Shriyan *et al.*<sup>[8]</sup>,

**Table 1: Distribution of 200 Cases According to Sex and Type of Wounds**

Type of Wound	Sex		Total
	Male	Female	
Postoperative wound infections	110 (71.42)	44 (28.57)	154
Burn wound infections	04 (13.79)	25 (86.20)	29
Bed sores	13 (76.47)	04 (23.52)	17
Total	127 (63.50)	73 (36.50)	200

**Table 2: Distribution of Pathogens from Nosocomial Wound Infections**

Name of organism	No. of isolates (n = 230)	Percentage
Staph. aureus	71	30.87
P. aeruginosa	63	27.39
Klebsiella spp.	31	13.48
Proteus spp.	21	09.13
E. coli	17	07.39
Citrobacter spp.	13	05.65
Acinetobacter spp.	09	03.91
Strep. pyogenes	05	02.17

**Table 3: Antibiogram of P. aeruginosa Isolated from Nosocomial Wound Infections**

Antimicrobial agent isolates	No. of susceptible	Percentage
Ciprofloxacin	28	44.44
Gatifloxacin	31	49.20
Gentamicin	25	39.68
Amikacin	41	65.07
Ceftazidime	29	46.03
Cefoperazone	34	53.96
Imipenem	62	98.41
Meropenem	36	57.14
Piperacillin	8	12.69

Al-Habib *et al.*<sup>[11]</sup> and Behesthi and Zia<sup>[9]</sup>. A monobacterial isolation rate of 82.30% and polybacterial isolation rate of 17.70% in the present study are not in agreement with earlier reports in which a lower rate of monobacterial and higher rate of polybacterial isolation have been reported<sup>[5,9]</sup>. It is likely that since at the time of collection of specimens precaution was taken to avoid the contaminating commensal flora and the efforts were made to obtain the pyogenic discharge from the sites of nosocomial wound infections, the bacterial isolates may represent only the bacteria which may have been etiologically related to the nosocomial wound infections. (Table 2) shows the distribution of pathogens isolated from nosocomial wound infections. Staph. aureus (30.87%), P. aeruginosa (27.39%) and Klebsiella spp. (13.48%) were the most common isolates.

Staph. aureus has been reported as most common isolate by various workers<sup>[5,8,10,12-17]</sup>. Giacometti *et al.* reported an isolation rate of 28.2%,<sup>[12]</sup> however Taiwo *et al.* reported isolation rate of 35.8%,<sup>[5]</sup> Dhar *et al.* reported 56.36%<sup>[13]</sup>, Nwachukwu *et al.* reported 42.30%<sup>[14]</sup>, Joyce and Lakshmidivi reported 33.3%<sup>[15]</sup>, Mohammad Imran *et al.* reported 25%<sup>[16]</sup>, Shriyan *et al.* reported 63%<sup>[8]</sup>, Goswami *et al.* reported 26.23%<sup>[17]</sup> and Ahmed reported 55%<sup>[10]</sup>. The findings of present study of isolation of Staph. aureus more frequently than other bacteria is consistent with these workers. The isolation rate of 30.87% of Staph. aureus is also more or less in agreement with Giacometti *et al.*<sup>[12]</sup>, Taiwo *et al.*<sup>[5]</sup>, Joyce and Lakshmidivi<sup>[15]</sup> and Goswami *et al.*<sup>[17]</sup>. However, it is very less as compared to Dhar *et al.*<sup>[13]</sup>,

Nwachukwu *et al.*<sup>[14]</sup>, Shriyan *et al.*<sup>[8]</sup> and Ahmed<sup>[10]</sup>, who have reported much higher rate of isolation of Staph. aureus in their study. P. aeruginosa has been reported as a most common agent by Arslan *et al.* (53%)<sup>[18]</sup>, Lari *et al.* (73.2%)<sup>[19]</sup>, Lilani *et al.* (23.52%)<sup>[6]</sup>, Mehta *et al.* (51.8%)<sup>[7]</sup>, Ruhil *et al.* (27.7%)<sup>[20]</sup>, Ranjan *et al.* (29.6%)<sup>[8]</sup>, Al-Habib *et al.* (50%)<sup>[11]</sup> and Behesthi and Zia (32.2%)<sup>[9]</sup>

The findings of the present study are in difference to these workers. The present study shows P. aeruginosa as a second most common agent. However, the isolation rate of 27.39% of P. aeruginosa in present study is more or less in agreement with Giacometti *et al.* (25.2%)<sup>[12]</sup>, Joyce and Lakshmidivi (24.4%)<sup>[15]</sup>, Ruhil *et al.* (27.7%)<sup>[20]</sup> and Ranjan *et al.* (29.6%)<sup>[8]</sup>. However, this isolation rate is much lower than Arslan *et al.* (53%)<sup>[18]</sup>, Lari *et al.* (73.2%)<sup>[19]</sup>, Mehta *et al.* (51.5%)<sup>[7]</sup> and Al-Habib *et al.* (50%)<sup>[11]</sup> and it is much higher than Shriyan *et al.* (9.5%)<sup>[8]</sup>, Ahmed (3%)<sup>[10]</sup> and Garba *et al.* (11%)<sup>[10]</sup>. These results show that the prevalence rate of nosocomial wound infections still remains very high. Staph. aureus and P. aeruginosa are the most important nosocomial pathogens causing nosocomial wound infections. (Table 3) shows the antibiogram of P. aeruginosa isolated from nosocomial wound infections. Imipenem (98.41%), amikacin (65.07%), meropenem (57.14%) and cefoperazone (53.96%) were found to be most effective antimicrobial agents. Gatifloxacin (49.20%), ceftazidime (46.03%), ciprofloxacin (44.44%) gentamicin (39.68%) and piperacillin (12.96%) were the other antimicrobial agents, which were found to be effective against <50% isolates.

Various workers have reported imipenem as the most effective antimicrobial agent<sup>[7,12,18,20,22,23]</sup>. Giacometti *et al.* reported 88.8% susceptibility to imipenem<sup>[12]</sup>, however Naqui *et al.* reported 77.3% susceptibility<sup>[23]</sup>. Mehta *et al.* reported 4.54% resistance to imipenem in *P. aeruginosa* from burn wound infections (95.36% susceptibility)<sup>[7]</sup>, however Ruhil *et al.* reported 76.9% susceptibility<sup>[20]</sup>. Imipenem has also been found highly effective against *P. aeruginosa* in a study carried out by Ranjan *et al.* (76.9%)<sup>[22]</sup>. However, it was found less effective in studies carried out by Behesthi and Zia (39%)<sup>9</sup> and Arslan *et al.* (44%)<sup>[18]</sup>. The finding of present study showing imipenem (98.41%) as most effective antibacterial agent is more or less consistent with these workers except for Behesthi and Zia (39%) and Arslan *et al.* (44%), who reported much less percentage of susceptibility, although imipenem was the most effective antimicrobial agent in their study also.

In the present study, the anti-pseudomonal penicillin, piperacillin (12.69%) was found to be least effective anti-pseudomonal agent. This finding is not in agreement with Al-Habib *et al.* who reported least resistance of *P. aeruginosa* to piperacillin with 60% resistance rate and 40% isolates susceptible to piperacillin<sup>[11]</sup>. Fluoroquinolones, gatifloxacin and ciprofloxacin in our study showed susceptibility rate of 49.20 and 44.44% respectively. These findings do not correlate with Taiwo *et al.* who reported ciprofloxacin (85.7%) as most effective agent followed by ofloxacin and pefloxacin (74.3%)<sup>[5]</sup>, Sule *et al.* reported ciprofloxacin (91.9%) as most effective agent<sup>24</sup>, Goswami *et al.* reported ciprofloxacin (83.78%) as most effective agent followed by gatifloxacin (51.35%)<sup>17</sup> and Ahmed *et al.* reported 100% susceptibility to ciprofloxacin<sup>[21]</sup>. Among the aminoglycosides used, amikacin (65.07%) was found to be second most effective agent in the present study. This finding is in agreement with Dhar *et al.*, who reported amikacin (65%) as most effective against *P. aeruginosa*. However, this finding is not in agreement with Lari *et al.*, who showed 90% resistance to amikacin<sup>[19]</sup>, Joyce and Lakshmidevi, who showed least sensitivity (9%) to amikacin<sup>15</sup> and Mohammed Imran *et al.*, who showed least susceptibility to amikacin (53%)<sup>[16]</sup>. Among the cephalosporins tested cefoperazone (53.96%) was found more effective than ceftazidime (46.03%). This finding is in contrast with Arslan *et al.* (1999), who reported ceftazidime more effective than cefoperazone<sup>[18]</sup>. In the present study, meropenem was found effective against 53.96%. This finding is similar to Goswami *et al.* (51.35%)<sup>[17]</sup>, but not in agreement with Mohammad Imran *et al.* (76%)<sup>[16]</sup>, Ruhil *et al.* (70.4%)<sup>20</sup> and Ranjan *et al.* (70.4%)<sup>[22]</sup>, who reported much higher rates of susceptibility to meropenem. The results of overall susceptibility patterns show that

there is a major difference in the results of susceptibility pattern of present study and other reports. This indicates that the susceptibility pattern changes from hospital to hospital, population to population and country to country. It also indicates the importance of study of susceptibility pattern, as emphasized by various international authorities that every hospital should have its own antimicrobial susceptibility pattern as the standard antibiotic susceptibility pattern may not hold true for every area/hospital.

**Summary:** Studies on bacteriology of nosocomial wound infections showed *Staph. aureus* (30.87%) as most predominant bacterium isolated and *P. aeruginosa* (27.39%) as second most predominant bacterium. *Strep. pyogenes* was the least common bacterium isolated. Antibiogram of *P. aeruginosa* from nosocomial wound infections showed imipenem (98.41%) as most effective agent and piperacillin (12.69%) as least effective agent.

## CONCLUSION

From the present study, it is concluded that: Nosocomial wound infections caused by *P. aeruginosa* continues to be an important problem to be thought seriously. Presence of *P. aeruginosa* at various environmental sources suggests that more aseptic care to be taken during the handling of hospitalized patients. Antimicrobial resistance remains a major clinical problem. The use of antimicrobial agents showing less activity in vitro and widely used antibiotics should be restricted for some time. Improper, unnecessary and indiscriminate use of antimicrobial agents encourage development of resistance. *P. aeruginosa* isolated from nosocomial wound infections, which are resistant to multiple antibiotics should be paid special attention. Common use of highly effective antimicrobial agents such as imipenem, meropenem, amikacin, etc. should be avoided and these agents should be used in clinical situations where no other antibiotic is effective

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