

Archives of Biotechnology and Pharmaceutical Research

<https://urfpublishers.com/journal/biotech-pharma-research>

Vol: 2 & Iss: 1

Prevalence of *Pseudomonas aeruginosa* in Wound Infections among Patients attending Selected Hospitals in Edo Central Senatorial District, Nigeria

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Citation: Iyevhobu KO, Eigbedion AO, Obohjemu OK. Prevalence of *Pseudomonas aeruginosa* in Wound Infections among Patients Attending Selected Hospitals in Edo Central Senatorial District, Nigeria. *Arch Biotech Pharma Res*, 2026;2(1):78-83.

Received: 10 March, 2026; **Accepted:** 27 March, 2026; **Published:** 31 March, 2026

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ABSTRACT

Wound infection is described as the deposition and multiplication of bacteria on a wound site causing an associated host reaction. The aim of this study was to determine antimicrobial resistance pattern of *Pseudomonas aeruginosa* and its prevalence in wound infection among patients attending selected hospitals in part of Edo Central Senatorial District, Edo State. A total of 152 pus/ wound swab samples were collected from both sexes and all aged patients. The wound swab samples were collected using Levine's technique and transported to a Microbiology Laboratory after collection in 0.5ml sterile normal saline solution for bacterial preservation. The wound swab samples were cultured by plating onto 10% Cetrimide Blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18-48 hours. Suspected colonies were further subcultured to obtain discrete colonies. This study has shown that the overall prevalence of *P. aeruginosa* among patients with wound infection was 60 (39.5%). The prevalence of *P. aeruginosa* was higher among subjects that attended ISTH (48%), who were 0-19years (66.7%) age range; Males (43.3%); with wounds at the abdomen (68.4%); wounds caused by burn (60%) and among Farmers (80.6%). This result showed a significant association between the prevalence of *P. aeruginosa* and subjects at ISTH;0-19years age range; Farmers, wounds at the Abdomen and are not significant among subjects who were Males and wounds caused by burns. *P. aeruginosa* was highly resistant to Gentamicin (81.7%) and Ofloxacin (61.6%). Doripenem (3.3%), Meropenem (6.7%), Ciprofloxacin (26.5%) and Imipenem (50%) antibiotics were found to be most effective in this study. According to this study, *P. aeruginosa* isolates were highly resistant to most of antimicrobial drugs used, which calls for an immediate action on the controlled use of antimicrobials in the hospitals and the need to monitor resistance.

Keywords: *Pseudomonas aeruginosa*, Wound, Infection, Patients, Resistance, Hospital

1. Introduction

Skin, the largest organ in the human body, plays a crucial role in the sustenance of life through the regulation of water and electrolyte balance, thermoregulation and by acting as a barrier to external noxious agents including microorganisms. However, when the epithelial integrity of skin is disrupted, a wound result¹. The skin is the largest external organ in the body that is in contact with the external environment². A wound can represent a simple or a severe disorder to an organ (such as the skin) or a tissue and can spread to other tissues and anatomical structures e.g., subcutaneous tissue, muscles, tendons, nerves, vessels and even to the bone. Wound is a break in the continuity of any bodily tissue due to violence, where violence is understood to encompass any action of external agency, including for example, surgery. The primary and major function of an intact skin is to protect the underlying tissue from colonization of potential pathogens that are present freely in the environment and skin surface. Therefore, following a wound, there is exposure of subcutaneous tissue leading to development of a nutritious, moist, warm and favorable environment for the colonization and multiplication of microorganisms³. Wounds can be acute or chronic. Examples of the acute wounds include surgical wounds, insect bites, burns, abrasions and cuts that can heal within a predictable time frame depending on the severity of the injury. However severe skin damage and wounds from gunshots or burns requires surgery debridement and antimicrobial therapy⁴. In contrast, the chronic wounds are mostly caused by internal mechanisms associated with an underlying predisposing disease such as diabetes or immune deficiency. Examples are leg and arterial ulcers, non-healing surgical wounds and diabetic foot ulcers⁵.

Infection occurs when a virulence factor expressed by one or more microorganisms in a wound conquers the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses⁴. Characteristic local responses are a purulent discharge or painful spreading erythema indicative of cellulitis around a wound⁴.

Infection is the main cause of delayed wound healing in primary closure, traumatic wounds, burns and chronic skin ulcers⁶. *Pseudomonas aeruginosa* is an opportunistic infectious pathogen that poses a management challenge while carrying significant morbidity and mortality⁷. Part of the challenge is the fact that excessive antibiotic use can promote the selection and multiplication of resistant isolates. *P. aeruginosa* is a ubiquitous nosocomial infection in burn patients and multi-drug-resistant strains are common in burn units, leading to a high mortality rate. It is the most common cause of bacteremia in burn patients and 14-33% of burn wounds are colonized with *P. aeruginosa* within 10 days of admission⁸.

Wound infection is described as the deposition and multiplication of bacteria on a wound site causing an associated host reaction⁹. Wound infection is the result of successful invasion and proliferation by one or more species of microorganisms, to a level that invokes a local and/or systemic response in the host, resulting in the formation of pus⁹. Burn wounds, surgical sites, bite wounds, acute soft tissue infections, diabetic foot ulcers and leg and pressure ulcer infections are all examples of wound infections⁹. Wound infection is a common complication

of wounds. It leads to delays in wound healing and increases the risk of loss of limb and life¹⁰. Implementation of effective strategies to prevent, diagnose and manage wound infection is important in reducing mortality and morbidity rates associated with such infection¹⁰.

Any wound is at risk of becoming infected. But the major risk factors for wound infection includes old age, prolonged hospital stay, immunocompromised state, indwelling devices like intravenous catheters, urinary catheters, eye, irrational use of antibiotics and other pre-existing conditions like malignancies, renal failure. Wounds expose components of tissue normally protected from the outside environment by skin or mucus membranes¹¹. Infection is one of the major barriers for the process of wound healing. Hence it has an adverse impact on the patient's quality of life¹¹. Wound infections continue to be a source of concern in clinical practice as they cause delayed or poor wound healing, which may lead to prolong hospital stays thereby incurring more cost of hospitalization⁷. Some microbes can produce a complex protective glycocalyx also called biofilm-which makes the infected wounds hard to be detected and treated⁷.

Wound infections are one of the most common hospitals acquired infections and are important cause of morbidity and account for 70-80% mortality¹². These wounds can be contaminated by bacterial pathogens thereby hampering the healing process and its management becomes resource demanding¹³. Bacterial wound infections are economically important because they can slow down the healing process, lead to wound breakdown, prolonged hospital stay and increase in the cost of treatment¹². High index of wound contamination with bacteria are the third most frequent nosocomial infections¹⁴. The contamination or mere presence of pathogenic organisms in wound without local or systemic tissue involvement may not result in infection; if the wound environment is conducive for bacterial growth. The organisms can cause destruction if left untreated¹⁵.

Most wound infections are caused by bacterial colonization, initiating either from the normal flora on the skin or bacteria from other parts of the body or the outside environment¹⁵. Some microbes can produce a complex protective glycocalyx also called biofilm-which makes the infected wounds hard to be detected and treated. Studies have repeatedly shown that *S. aureus*, *P. aeruginosa* and *Proteus* spp are the most common bacteria found in wound infections in Nigeria¹⁶.

Pseudomonas aeruginosa is the most important of specie of *Pseudomonas* genus. It's a Gram-negative rod, strict aerobic organism. *P. aeruginosa* is oxidase positive¹⁷. *Pseudomonas aeruginosa* is an opportunistic infectious pathogen that poses a management challenge while carrying significant morbidity and mortality¹⁷. *P. aeruginosa* can cause a variety of skin infections, such as infections of wound, infection of nail, infected toe web, pseudomonal folliculitis and pseudomonal cellulitis¹⁸. Infection of burn wounds is the most common recognized condition caused by *P. aeruginosa*. Pseudomonal wound infection is characterized by the presence of dark brown eschar associated with edema and hemorrhagic necrosis¹⁹. *Pseudomonas* skin infections are commonly seen in patients who are exposed to moisture¹⁸. *P. aeruginosa* has a high intrinsic and acquired antibiotic resistance²⁰. Part of the challenge is the fact that excessive

antibiotic use can promote the selection and multiplication of resistant isolates which makes its treatment challenging¹⁹. *P. aeruginosa* infected wound is characterized by a significantly greater area and a delayed or prevented healing process²¹. *P. aeruginosa* is localized in the deepest region of wound bed; the organism often causes biofilm-based chronic wound infections which may suppress leukocyte activity¹⁷.

Wound Infections are mutual and costly complications that increase morbidity and mortality in hospitalized patient. Most wound pathogens are bacteria and the etiology of wound infection in Nigeria follows a similar trend as in other countries⁶. In Edo state, wound infection analysis has revealed various findings across different areas in the state, emphasizing the need for local prevalence and susceptibility investigations. Despite this, studies have repeatedly shown that *P. aeruginosa*, *S. aureus* and *Proteus* spp are the most common bacteria found in wound infections in Nigeria¹⁶.

Currently, *Pseudomonas aeruginosa* is one of the most commonly isolated antimicrobial resistant pathogens worldwide²². However, there is insufficient studies and research on the prevalence of *Pseudomonas aeruginosa* in wound infection in Nigeria. Therefore, this research was aimed at the identification of isolates of *Pseudomonas aeruginosa*, obtaining the antibiotic susceptibility pattern and determining the prevalence of *Pseudomonas aeruginosa* isolates from the various wound samples collected from patients within Edo Central Senatorial District of Edo State.

2. Materials and Methods

2.1. Study area

This study was carried out in Esan Central and Esan West. Edo State. Esan Central is a Local Government Area of Edo State, Nigeria. Its administrative headquarters is located in the town of Irrua and it has an area of 253km² and a population density of 545.1/km². Esan West Local Government Area has its headquarters in the town of Ekpoma and it lies between latitude 6° 43' and 6° 45' North of the equator and longitudes 6° 6' and 6° 8' East of the Greenwich Meridian. It has an area of 502km² and a population density of 333.3/km². It has an estimated population of over 190,000 people which consists of an adult male population of over 60,000 and adult female population of over 60,000.

2.2. Research design

This study adopted the cross-sectional design to determine the prevalence of *Pseudomonas aeruginosa* isolated from wound infection and its antibiotic susceptibility pattern to selected antimicrobial agents. Consent from the patients was sought for before their samples were collected for this research work.

2.3. Sample size

The sample size is computed using the formula:

$$N = Z^2 pq / d^2$$

n=sample size

Z=statistic for level of confidence

p=estimated prevalence

d=precision

Considering 86.133% estimated prevalence (p) of wound infection in Okolobiri, Bayelsa state by Kemebradikumo et al., (2013), 5% precision (d=0.05) and 95% level of confidence (Z=1.96) The sample size was estimated;

q-Alternative proportion (1-p) which is 1-0.8613=0.1387 d-Precision (0.05)

$$N = \frac{(1.96)^2 \times 0.8613 \times (0.1387)}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.8613 \times 0.1387}{0.0025}$$

$$N = 183.57$$

$$N = 183.57$$

A total of 152 wound specimens were requested from consenting 152 patients of both sex and all age groups.

Target Population

The target population for the study include in-patients and out-patients attending four selected hospitals in Edo state namely: Irrua Specialist Teaching Hospital (ISTH), Eromonsele Hospital, General Hospital Ekpoma, Eseho Medical Center, Ekpoma

2.4. Sample collection and transportation

Wound swabs were collected from one hundred and fifty-two (152) wound cases with the assistance of the nurses randomly using commercially available sterile cotton swabs. The samples were preserved temporarily in sterile normal saline and then transported to the microbiology laboratory.

2.5. Isolation of organism

The preserved specimens were plated on MacConkey agar and Cetrimide blood agar plates using the streak plate method and incubated aerobically at 37°C for 24-48 hours.

2.6. Characterization and identification of pseudomonas aeruginosa isolates

This was done to identify the presumptive *Pseudomonas aeruginosa* isolates obtained from the different wound specimens analyzed. Characterization was done phenotypically on the basis of their colonial morphology, microscopy (Gram stain) and biochemical attributes observed after performing catalase test, oxidase test and citrate test for each of the isolates.

2.7. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of all identified isolates was done according to the criteria of the Clinical and Laboratory Standards Institute method²³. Briefly, section of the *Pseudomonas aeruginosa* isolate was taken and transferred to a tube containing 3 ml of sterile peptone water and was mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the density of a McFarland 0.5 in order to standardize the inoculum size. A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of nutrient agar. The inoculated plates were left at room temperature to dry for 3-5 minutes. With the aid of sterile forceps, the following concentration of antibiotic discs was put on the surface of nutrient agar; Gentamicin (10µg),

Ciprofloxacin (5µg), Ceftazidime (30µg), Cefuroxime (30µg), Ofloxacin (2µg), Meropenem (10µg), Imipenem (10µg) and Doripenem (30µg). The criteria used to select the antimicrobial agents tested was based on their availability and frequent prescriptions for the management of *Pseudomonas aeruginosa* infections in the study area. The plates were then incubated at 37° for 48 hours²³. Diameters of the zone of inhibition around the discs was measured using a digital caliper and the isolates was classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI²³.

2.8. Method of data analysis

Data generated were analyzed using Microsoft Excel Software and Statistical calculators Data analyze significant difference at 95% confidence interval. A p-value < 0.05 was considered statistically significant were presented as frequency and percentage.

3. Results

Table 1 shows the distribution of *P. aeruginosa* among patients with wound infection in relation with the selected Hospitals. The highest prevalence of *P. aeruginosa* were among subjects at ISTH (48%), followed by General Hospital (25%) and other Hospitals (20%). The overall prevalence of *P. aeruginosa* among patients with wound infection in relation with the selected Hospitals was 60 (39.5%). However, the difference was statistically significant ($P \leq 0.05$).

Table 2 shows, the highest prevalence of *P. aeruginosa* among patients with wound infection based on age range were among 0-19 (66.7%), followed by subjects within 30-39 (50%) and 50-59 (43.8%). The result shows there's a significant association between the prevalence of *P. aeruginosa* and age range. The overall prevalence of *P. aeruginosa* among patients with wound infection based on age range was 60 (39.5%).

Table 3 shows the highest prevalence of *P. aeruginosa* among patients with wound infection based on gender was among the Male (43.3%) compared to the Female (33.9%). The overall prevalence of *P. aeruginosa* among patients with wound infection based on gender was 60(39.5%). However, this difference was statistically not significant ($P > 0.05$).

As shown in **Table 4**, the highest prevalence of *P. aeruginosa* among patients with wound infection based on site of wound-Abdomen (68.4%), followed by the Arm (57.1%) and Thigh (37.5%). The overall prevalence of *P. aeruginosa* among patients with wound infection based on the site of wound was 60 (39.5%). However, this difference was statistically significant ($P \leq 0.05$).

Table 5 shows the highest percentage of *P. aeruginosa* among patients with wound infection based on the causes of wound-Burn (60%), followed by Surgery (44.4%). The overall prevalence of *P. aeruginosa* among patients with wound infection based on the causes of wound was 60 (39.5%). However, this difference was statistically not significant ($P > 0.05$).

The perception of health care workers towards interdisciplinary approach to patient care was found to be positive. This goes on to say that working as a team is highly appreciated. The attitudes of healthcare workers towards interdisciplinary approach to patients care in Asaba specialist hospital was found to be highly positive.

Table 1: Distribution of *P. aeruginosa* among patients with wound.

Hospital	No. of Samples Examined (%)	No. of Bacteria Isolated (%)
ISTH	100	48(48)
General Hospital	32	8(25)
Other Hospital	20	4(20)
Total (%)	152	60(39.5)

$$X^2 = 9.023; p - \text{valn } e = 0.0109$$

Table 2: Distribution of *P. aeruginosa* in Wound Infection Based on Age Range.

Age (years)	No. of Samples Examined (%)	No. of Bacteria Isolated
0-19	15	10(66.7)
20-29	39	11(28.2)
30-39	40	20(50)
40-49	28	7(25)
50-59	16	7(43.8)
≥60	14	5(35.7)
Total (%)	152	60(39.5)

$$X^2 = 11.231; p - \text{Value} = 0.0469$$

Table 3: Distribution of *P. aeruginosa* in Wound Infection Based on Gender.

Gender	No. of Samples Examined (%)	No. of Bacteria Isolated (%)
Male	90	39(43.3)
Female	62	21(33.9)
Total (%)	152	60(39.5)

$$X^2 = 1.376; p - \text{valn } e = 0.2407$$

Table 4: Distribution of *P. aeruginosa* in Wound Infection from different site of Wound.

Site of Wound	No. of Samples Examined (%)	No. of Bacteria Isolate (%)
Leg	86	29(33.7)
Arm	7	4(57.1)
Abdomen	19	13(68.4)
Thigh	16	6(37.5)
Others	24	8(33.3)
Total (%)	152	60(39.5)

$$X^2 = 9.175; p - \text{Value} = 0.0568$$

Table 5: Distribution of *P. aeruginosa* in Wound Infection Based on the causes of Wound.

Causes of Wound	No. of Samples Examined (%)	No. of Bacteria Isolated
Accident	98	35(35.7)
Surgery	36	16(44.4)
Burn	10	6(60)
Others	8	3(37.5)
Total (%)	152	60(39.5)

$$p - \text{value} = 0.4353 \quad X^2 = 2.729$$

4. Discussions

Wound Infections are mutual and costly complications that increase morbidity and mortality in hospitalized patient.

Most wound pathogens are bacteria and the etiology of wound infection in Nigeria follows a similar trend as in other countries. In Edo State, wound infection analysis has revealed various findings across different areas in the state, emphasizing the need for local prevalence and susceptibility investigations. Studies have repeatedly shown that *P. aeruginosa* is the most common bacteria found in wound infections in Nigeria. Despite this problem, studies investigating the antimicrobial resistance pattern of *Pseudomonas aeruginosa* and its prevalence in wound infection are scarce. Hence, this study sought to investigate the antimicrobial resistance pattern of *Pseudomonas aeruginosa* and its prevalence in wound infection in Edo State.

P. aeruginosa is an important Gram-negative pathogen, particularly in patients at risk for nosocomial infections. *P. aeruginosa* is the second most common pathogen isolated from chronic wound infections globally. The prevalence and sensitivity of *P. aeruginosa* often varies between communities, hospitals in the same community and among different patient population in the same hospital. Faced with these variations, the Medical Laboratory Scientists has the responsibility of making clinical research and should have access to recent data on the prevalence and antimicrobial resistance pattern of these commonly encountered pathogens. It is therefore important to institute a system for the surveillance of antimicrobial resistance that will involve the collection and collation of both clinical and microbiological data.

P. aeruginosa is an opportunistic human pathogen. It is “opportunistic” because it seldom infects healthy individuals. *P. aeruginosa* was the second most abundant organism. However, *P. aeruginosa* was the most commonly isolated organism in the studies that corresponded to Nigeria²⁴. In this study, the findings demonstrated the predominance of *Pseudomonas aeruginosa* among young age groups i.e. 0-19 (66.7%) and these results were in agreement with results from previous studies conducted within Nigeria^{25,26}.

In this study, it was discovered that the prevalence was higher among the Male gender (4.3%) 58% of *P. aeruginosa* isolates among Male Patients^{26,27}. The highest percentage of *P. aeruginosa* among patients with wound infection based on occupation was among Farmers (80.6%), in this study and it was statistically significant. Similarly, *P. aeruginosa* isolates were observed to be predominant among farmers in another study conducted in South-West, Nigeria²⁸. This is believed to be based on their exposure to rural areas and nature of work²⁸.

Generally, antibiotic susceptibility of *P. aeruginosa* isolates to the commonly used antibiotics was low; Gentamicin (81.7%) and Ofloxacin (61.6%) were the most highly resistant antimicrobial agents while the Doripenem (3.3%), Meropenem (6.7%), Ciprofloxacin (26.6%) and Imipenem (50%) antibiotics were found to be the most effective antimicrobial agents observed in this study. Other reports in Nigeria, confirms that the susceptibility rate of *P. aeruginosa* isolates to the eight antimicrobials tested in vitro were relatively low compared to the sensitivity pattern to different anti-pseudomonal drugs reported worldwide^{29,30}. Good antimicrobial use is necessary for effective wound management. A study by Shashikala, et al.³⁰ confirms that *P. aeruginosa* resistance has been rising to a point where approximately 40% of the isolates are resistant to ‘antipseudomonal’ drugs.

5. Conclusion

In conclusion, this study shows a significant resistance of *P. aeruginosa* isolates to the commonly used antimicrobial agent with a high prevalence among the Male gender and Farmers. The multiple resistance of *P. aeruginosa* for an immediate action on the controlled use of antimicrobials agents in the hospitals and the need to monitor resistance. According to the World Health Organization (WHO), the management of *Pseudomonas aeruginosa* in wound infections includes the following recommendations:

Prompt and accurate diagnosis by Medical Laboratory Scientists in identifying *Pseudomonas aeruginosa* in wound infections through appropriate laboratory testing

Proper wound care practices, including regular cleansing and debridement, should be implemented to prevent and manage *Pseudomonas aeruginosa* infections.

Public awareness should be enforced especially to those who have little to no educational background in order to aid health promotion and prevention of antimicrobial drug resistance.

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