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Research Article

Prevalence and Antibiotics Susceptibility Profile of *Pseudomonas aeruginosa* Associated with Wound Infections in Port Harcourt, Nigeria

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Abstract

Infection of wounds is a common, often severe and costly complication resulting from bacterial colonization. The aim of this study was to investigate the prevalence and antibiotics susceptibility pattern of *Pseudomonas aeruginosa* in wounds of patients attending a tertiary hospital in Port Harcourt. One hundred and fifty (150) samples from wounds in different hospital wards were collected by using sterile cotton swab sticks. The specimen were taken to the Microbiology Laboratory, Rivers State University, Port Harcourt, Nigeria, for microbiological analysis, using Standard procedures for cultural characterization. Antibiotics sensitivity was evaluated using the Kirby-Bauer disc diffusion method. Data showed that Burns had the highest percentage occurrence of 24%, followed by Caesarean section (21%). Scrotal wounds had a percentage occurrence of 17%. Accident and Emergency (A/E) and Plastic Surgery both had a percentage occurrence of 10% each, while traumatic wounds and surgical wounds had a percentage occurrence of 7% each. The least occurrence was seen in Dermatomytosis Ward (DMS) with percentage occurrence of 4%. The study further revealed that the highest incidence of wound infection (55%) was associated with the female gender compared to the male gender having a percentage occurrence of 45%. The antimicrobial susceptibility test showed that 96.30% of the isolates were resistant to Cefuroxime, while 92.59% and 85.19% of the isolates were resistant to Augmentin and Cefixime, respectively. In the case of Gentamycin, there was a moderate level of resistance, with 51.85% of the isolates being resistant. Ceftazidime and Ofloxacin both had lower resistance profile, with 48.15% of the isolates showing resistance. The highest growth inhibition was seen in Ciprofloxacin, with only 37.04% of the isolates showing resistance. The high level of antibiotics resistance by *Pseudomonas aeruginosa* associated with wound colonization in this study is of a serious concern. Therefore the need for proper wound management in the hospital environment cannot be overemphasized.

Keywords: Antibiotics Susceptibility; Prevalence; *Pseudomonas aeruginosa*; Wound Infection; Port Harcourt

Introduction

Wound injuries are serious public health problems worldwide [1]. Infection of wounds is a common, often severe and costly complication resulting from bacteria colonization. Traumatic ulcers can become a serious complication resulting from *Pseudomonas* infection. They are now the most common proximate and nontraumatic causes of leg amputation [2]. Various microorganisms colonize the wound and in some patients one or more species of organisms proliferate in the wound, which may lead to tissue damage, host response accompanied by inflammation, and other manifestations wound infection [3]. Wound make available a moist, warm and nutritive environment conducive to microbial colonization, proliferation, and infection [4].

Several bacterial species live on human skin, in the nasopharynx, gastrointestinal tract, and other parts of the body with little potential for causing disease, because of the first line of defense

within the body [5]. Despite this, any breach in the skin surface whether trauma, accident, surgical operation, or burn provides an open door for bacterial infections [2]. The most common underlying event for all wounds is trauma [3]. Trauma may be accidental or intentionally induced. The category of intentionally induced trauma includes hospital-acquired wounds, which can be grouped according to how they are acquired, such as surgically and by using intravenous medical devices. The non-intentionally induced, hospital-acquired wounds can be the pressure sores [6]. The development of wound infection depends on the integrity and protective function of the skin, the number and types of organism and their synergy, the pathogenicity and virulence of the bacterial species, nature of surgery, use of antibiotics, and the immunocompetency of the host [7]. Wounds that are infected are characterized by bacterial burden, chronic inflammation, and an unbalanced cellular defense mechanism [7].

Pseudomonas aeruginosa is an important human opportunistic bacterium in wounds. It is a gram-negative aerobic, rod-shaped non- lactose fermenting bacterium with unipolar motility [8].

P. aeruginosa is often preliminarily identified by its pearlescent appearance and grape-like or tortilla-like odour *in vitro*. It can be responsible for a spectrum of presentations from superficial colonization of ulcers to extensive tissue damage, including osteomyelitis, septic arthritis and bacteremia [9]. *P. aeruginosa* and *Staphylococcus aureus* are the most commonly isolated organism from diabetic ulcer [10]. *Pseudomonas aeruginosa* is a ubiquitous Gram-negative bacterium belonging to the family *Pseudomonadaceae* that is able to survive in a wide range of environments [10]. The organism is an epitome of opportunistic nosocomial pathogen, which causes a wide spectrum of infections and leads to substantial morbidity in immune compromised patients. Despite therapy, the mortality due to nosocomial *Pseudomonas* sp is approximately 70% [6]. Unfortunately, *Pseudomonas aeruginosa* develops resistance to most of antibiotics [11], thereby jeopardizing the selection of appropriate treatment options. *Pseudomonas* sp is resistant to Beta-lactams, including broad-spectrum cephalosporins, quinolones, chloramphenicol and tetracyclines majorly, because of the very low permeability of their cell wall. Moreover, *P. aeruginosa* is characterized by the production of inducible cephalosporinase, active efflux *and* poor affinity for the target (DNA gyrase), three mechanisms that synergize with poor cell wall permeability [6]. Polymicrobial infections predominate in severe wound infections, as observed in a study in which E*scherichia coli* was noted to be the most prevalent bacteria isolated with a prevalence of 18.6%, with *Staphylococcus aureus* having 10.8% occurrence, followed by *Pseudomonas* spp. (8.3%). In a previous study by Kokis., *et al.* (2005) [12], *Staphylococcus aureus* was isolated in 48.46% of cultures, *Pseudomonas aeruginosa* (16%) and *Klebsiella* (13.85%) [12]. Several researchers have reported *Staphylococcus aureus* in 38.4% of cultures from diabetic ulcers, *Pseudomonas aeruginosa* in 17.5% and *Proteus mirabilis* in 14% [13]. The aim of the study therefore was to evaluate the prevalence and antibiogram of *Pseudomonas aeruginosa* in wound infections in Port Harcourt, Nigeria.

Materials and Methods

Study area

The study was carried out in University of Port Harcourt Teaching Hospital (UPTH) Choba, Rivers State, Nigeria. The hospital is located at $4^0 53' 58$ N6^0 55'43^0E coordinates. The health facility was established in 1979. The 250 bed hospital has an average admission rate of 1,500 patents. The hospital serves the city of Port Harcourt, the Rivers State capital with a population of 1.5million and is a referral hospital for most of the other hospitals and clinics in the city and surrounding towns and villages in the entire Rivers State.

Collection of samples

One hundred and fifty (150) wound specimens from eight (8) wound types in the hospital wards (Accidents and Emergency, Caesarean Section(C/S), Scrotal Wounds, Surgical wounds, Burns, Traumatic wounds, Diabetic Foot Ulcers (DFU), Dermatomytosis Ward (DMS) and Plastic surgery) were collected by using sterile cotton swabs. The distribution of the specimens in this study included 80 Males and 70 Females, 16 Accident and Emergency, 1 scrotal wound specimen, 38 Burns, 22 Caesarean section (C/S), 4 Dermatomytosis, 4 Diabetic Foot Ulcer, 18 Plastic Surgery, 6 Surgical wounds and 23 Traumatic wound specimens. The specimen were transported to the Microbiology Laboratory, Rivers State University, Port Harcourt, Nigeria. The samples were preserved in ice-packed container while on transit and analyzed within two hours of collection.

Isolation of *Pseudomonas aeruginosa*

The streak plate technique was used for the isolation of *Pseudomonas aeruginosa*. The specimens were streaked on the surface of freshly prepared sterile Nutrient agar plates and Cetrimide agar plates. The inoculated plates were incubated at 37°C for 24 hours. The pure cultures of isolates were obtained by sub-culturing onto newly prepared Nutrient agar plates.

Characterization and identification of isolates

A sterile wire loop was used to sub culture the organisms for purification onto nutrient agar and incubated at 37^{0}C for 24h. Individual colonies were characterized on the basis of their colony morphology, microscopic examination and biochemical characteristics. Identification of bacterial isolates were then determined by comparing their characteristics with those of known taxa as described in Bergy's Manual of Determinative Bacteriology [14].

Test for Biofilm Production

The tube method was used as described by Christensen., *et al.* (1995) [15]. This is a qualitative method for biofilm detection. A loopful of the test organism was inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37^{o}C for 24 h. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilm formed were scored as 1-weak/none, 2-moder-ate and 3-high/strong.

Haemolysis test

Pseudomonas aeruginosa isolates were subcultured on freshly prepared 5% blood agar and incubated in the incubator at 37^o C for 24 hours. The presence and type of blood lysing (haemolysis) was observed after a 24 hour incubation.

Standardization of bacteria culture

A loop full of test organism was inoculated into nutrient broth and incubated for 24 hours. About 0.2 ml from the 24 hours culture of the organisms was added into 20 ml sterile nutrient broth and incubated for 3-5 hours to standardize the culture to 0.5 McFarland standards (10^6 CFU/ml) prior to use [16].

Antibiotics susceptibility test (AST)

Kirby Bauer sensitivity test was employed. Zero point one Millilitre (0.1 ml) of the standardized culture was aseptically inoculated into a 20 ml molten Mueller Hinton agar and gently swirled to effect mixing. A sterile forceps was used to implant the commercial multi-antibiotics disc onto the surface of the medium" aseptically. The culture was incubated at 37 ^{0}C for 24 hours. After the incubation period, Petri dishes were examined and the zone of inhibition across the various antibiotics was determined. Standard antibiotics used included: Ceftazidime (CAZ), Cefuroxime (CRX), Gentamycin (GEN), Cefixime (CXM), Ofloxacin (OFX), Augumetin (AUG), Nitrofirantoin (NIT) and Ciprofloxacin (CPR).

Multiple antibiotics resistance (MAR)

The Multiple Antibiotics Resistance (MAR) Index was determined using the formulae:

A/B

Where:

A= The number of antibiotics the organisms were resistant to.

B= The number of antibiotics tested or used in the study.

Results

Percentage occurrence of *Pseudomonas aeruginosa* in wards

The percentage occurrence of *Pseudomonas aeruginosa* isolated from wards is shown in figure 1. The percentage of occurrence ranged from 7%-24%. Burns indicated the highest percentage occurrence of 24% which was followed by Caesarean Section with 21%. Trailing behind Caesarean Section was scrotal wounds with a percentage occurrence of 17%. Accident and Emergency (A/E) and Plastic Surgery both had a percentage occurrence of 10% each while traumatic wounds and surgical wounds also had a percentage occurrence of 7% each as shown in figure 1.

Percentage occurrence of *Pseudomonas aeruginosa* among gender

The percentage occurrence of *Pseudomonas aeruginosa* among the males and females in different wards is shown in Figure 2. The percentage occurrence ranged from 45% - 55%. The highest percentage occurrence of 55% was seen in the female gender while the least percentage occurrence of 45% was seen in males (Figure 2).

Percentage of biofilm producing *Pseudomonas aeruginosa*

The percentage occurrence of biofilm producing and non-biofilm producing *Pseudomonas* sp was evaluated. The percentage occurrence ranged from 4% - 96%. Biofilm producing *Pseudomonas* sp had the highest percentage occurrence of 96% while non-biofilm producing *pseudomonas* sp had the least percentage occurrence of 4% (Figure 4).

Haemolysis test results

A complete hemolysis was seen for all *Pseudomonas aeruginosa* isolates. It is indicative that the organism produces hemolysin a toxin that attacks red blood cells. It also confers virulence of *Pseudomonas aeruginosa*.

Antimicrobial susceptibility of *Pseudomonas aeruginosa*

Antimicrobial susceptibility test with standard antibiotics was carried out on the *Pseudomonas aeruginosa* isolates. The percentage of resistance to antibiotics ranged from 37.04% - 96.30%. The highest percentage resistance was seen in Cefuroxime with a percentage of 96.30%. This was followed by Cefixime and Augumentine with percentage resistance of 92.59% each, trailing behind Cefixime and Augumentine was Nitrofirantion with percentage resistance of 88.88%. Gentamycin had a percentage resistance of 51.85% followed by Ceftazidime and Ofloxacin with 48.15% each. The least percentage resistance was seen in Ciprofloxacin with percentage resistance of 37.04%. The percentage of susceptibility to standard antibiotics used ranged from 3.70% - 40.74%. The Least percentage sensitivity was seen in Cefuroxime with 3.70%, followed by Augumetine and Cefixime which had 7.41% each. This was followed by Nitrofirantoin with 11.11%. A percentage sensitivity of 33.33% was seen in Ciprofloxacin while a percentage sensitivity of 37.04% was seen in Gentamycin. The highest percentage of sensitivity was seen in Ceftazidime, with percentage sensitivity of 40.74% (Table 1).

Multiple antibiotics resistance (MAR) index of *Pseudomonas aeruginosa* isolated

The MAR index values ranged from 0 – 1. Data revealed the MAR index of 0.9 was the most frequent, as it was associated with 29.63% of the isolates, followed by MAR index 0.5, associated with 25.93% of the isolates. MAR index 1 had a percentage occurrence of 22.22% and MAR index 0.6 had a percentage occurrence of 11.11% (Figure 4).

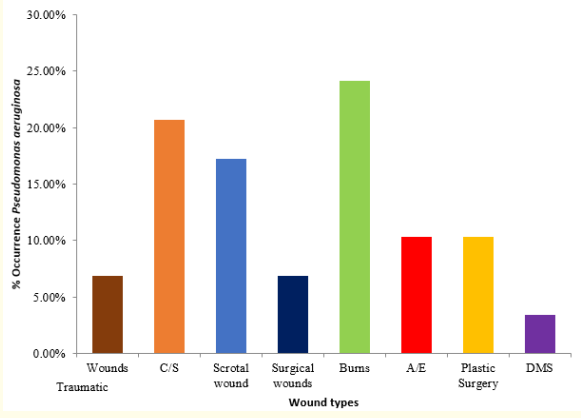

Figure 1: Percentage Occurrence of *Pseudomonas aeruginosa* in hospital wards.

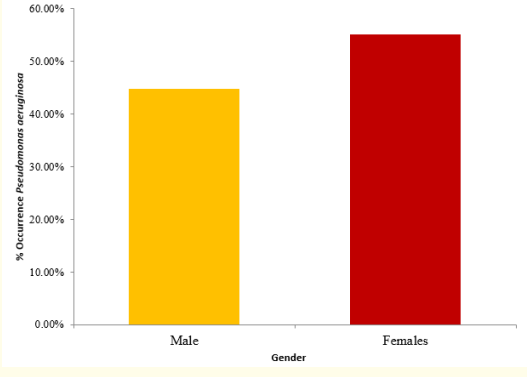

Figure 2: Percentage occurrence of *Pseudomonas aeruginosa* among males and females from the different wards.

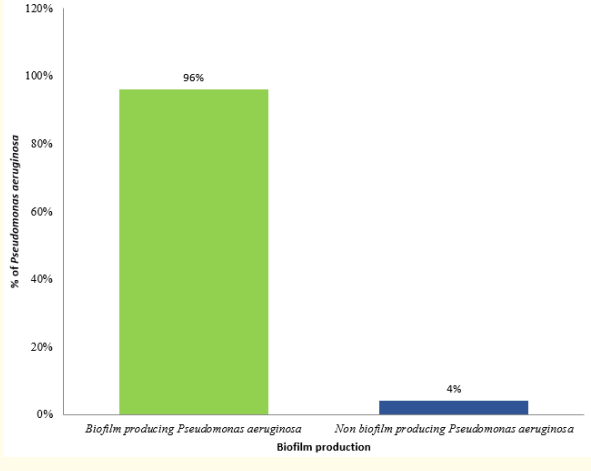

Figure 3: Percentage Occurrence of Biofilm producing and Non-biofilm producing *Pseudomonas aeruginosa.*

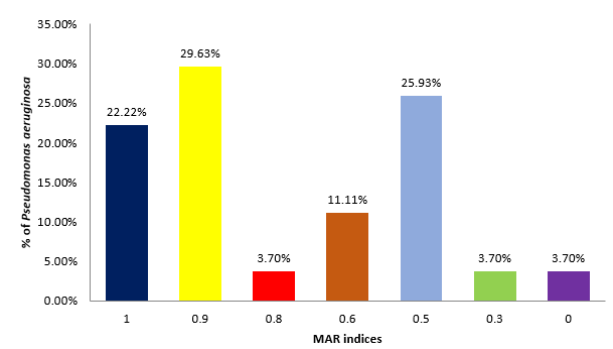

Figure 4: Mar index of *Pseudomonas aeruginosa* Isolated from wounds.

S/N	SAMPLE	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR
Number of R (%)		13(48.15)	26(96.30)	14(51.85)	23(85.19)	13(48.15)	25(92.59)	21(77.78)	10(37)
Number of S (%)		11(40.74)	1(3.70)	10(37.04)	4(14.81)	10(37.04)	2(7.41)	6(22.22)	9(33.33)
Number of I (%)		3(11.11)	0(0.00)	3(11.11)	0(0.00)	4(14.81)	0(0.00)	0(0.00)	8(29.63)

Table 1: Antibiotics Susceptibility Profile of *Pseudomonas aeruginosa*.

KEY: R = Resistant, S = Sensitive, I = Intermediate, CAZ = Ceftazidime, CXM = Cefixime, GEN = Gentamycin, CPR = Ciprofloxacin, CRX = Cefuroxime, OFX = Ofloxacin, AUG = Augmentin, NIT = Nitrofirantoin

Discussion

Percentage occurrence of *Pseudomonas aeruginosa*

Data obtained from the study revealed that burns had the highest percentage occurrence of *Pseudomonas aeruginosa,* while the least occurrence was associated with Dermatomytosis ward. These could be as a result of the fact that wounds from surgical site are exposed to many ubiquitous environmental pathogens which include *P. aeruginosa* found in unsterile surface, water and soil. These findings had been reported by Balarjishvili., *et al.* (2015) [17]. The pattern of colonization of surgical wounds by *P. aeruginosa* was observed in this study, which showed a prevalence of 29.6%. The source of this pathogen may not be unconnected with nosocomial infections as *P. aeruginosa* is known to be one of the most common pathogen causing wound infection usually in developing countries, as well as an epitome of opportunistic nosocomial pathogen, which causes a wide spectrum of infection and lead to substantial morbidity in immune compromised patients [17].

Immediately colonization and infection are established, *P. aeruginosa* becomes one of the worst pathogens of human and it is known to have intrinsic multi-drug resistance capabilities (Wong., *et al.* 2015). It has a large genome of about 6.3 million base pairs (bp), house 8 virulence genes, the large genome size increase the probability of possible mutation sites and thus gives reasons for its virulence versatile, its growing multi-drug resistance and the high mortality rate associated with its infection [17].

Antimicrobial susceptibility test of *Pseudomonas aeruginosa*

In the study, antimicrobial susceptibility test with standard antibiotics was carried out on the *Pseudomonas* species isolated. Ceftazidime being observed to inhibit the growth of most of the isolates in the study suggests the drug promises to be a potent first line drug for the treatment of *Pseudomonas aeruginosa* infections. The study indicated a high resistance of the isolates to most of the antibiotics used with 96.30% of the isolates resistant to Cefuroxime. The high resistance could be indicative of inappropriate use or abuse of antibiotic by the patients. The resistance could also be plasmid related. *Pseudomonas aeruginosa* develops resistance to most of antibiotics. The organism is naturally resistant to Beta-

lactams, including broad-spectrum cephalosporins, quinolones, chloramphenicol and tetracyclines majorly, because of the very low permeability of their cell wall [6,18].

According to Munita and Arias (2016) [19], *P. aeruginosa* can obtain antibiotic resistance as a result of mutation or as result of acquiring genes which are resistant through horizontal gene transfer. Furthermore, *P. aeruginosa*, generate resistance via different mechanism, making the elimination of the pathogen difficult, resulting to more cases of continuous infections [20].

The results obtained from the study is similar to a study by Walcott., *et al*. (2015) [21] where it was observed that an antibiogram of *P. aeruginosa*, showed that 39% of the *Pseudomonas* sp was found to be resistant to Ciprofloxacin, Ofloxacin, Pefloxacin and Sparfloxacin.

Multiple antibiotics resistance index of *Pseudomonas aeruginosa*

In the study, the MAR index ranged from 0 - 1. The MAR index 0.9 had the highest percentage frequency, being associated with 29.63% of the isolates. A MAR index greater than 0.2 indicates source of contamination is from where antibiotics are frequently used. This could justify the high level of drug resistance by the *Pseudomonas* sp isolated in the study. Similar MAR index had been reported by previous researchers [22] who suggested that the resistance of most *Pseudomonas* species to some antibiotics may be largely due to the presence of the resistance ampC gene, presence of heavy plasmids amongst other factors. This is in agreement with the findings that *P. aeruginosa* is a classic opportunistic pathogen especially because of its innate resistance to many antibiotics and its acquired resistance due to plasmid mediation [2].

Conclusion

There is an increase in the rate of clinical infection as a result of multidrug resistant strains of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was isolated from wound infection among patients in University of Port Harcourt Teaching Hospital in this study. Burns indicated the highest percentage occurrence while traumatic wounds and surgical wounds had equal percentage occurrence among the wards examined. The highest percentage occurrence was seen in the female gender.

There was a high level of drug resistance by the *Pseudomonas aeruginosa* isolated in the study. Ceftazidime showed promising potentials in the treatment of *Pseudomonas aeruginosa* infections in wound patients in hospitals.

The study has revealed that *Pseudomonas* aeruginosa was frequently associated with wound colonization with associated high level of antibiotics resistance. This therefore, necessitates the need for proper wound management using aseptic procedures as well as the right antibiotics against this pathogen.

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