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Antibiotic Susceptibility Studies of *Pseudomonas aeruginosa* Isolated from Wounds of Patients Attending General Hospital Minna, Nigeria

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Abstract

This work was carried out to determine the antibiotic Susceptibility profile of *Pseudomonas aeruginosa* isolated from wounds of patients attending General Hospital Minna Niger State. A total of 100 wound swab samples were collected from July to September, 2015 and subsequently analyzed. Isolation and identification of *Pseudomonas aeruginosa* was carried out using Cetrimide agar and subjected to series of biochemical tests. Antibiotic susceptibility tests were carried out on the isolates. One way Anova was used to statistically analyze the data. The bacteria isolated and identified from patient wounds includes, *Pseudomonas aeruginosa*, 53(86.88%), *Pseudomonas fluorescens*, 6(9.83%), and *Pseudomonas sp*, 2(3.29 %). *P. aeruginosa* showed susceptibility to Ciprofloxacin (94.3%), Tarivid (86.8%), and Augmentin (73.5%), but resistant to Streptomycin and Ampicillin. *P. fluorescens* exhibited Susceptibility to Ciprofloxacin (83.3%) and showed 100% resistance to Gentamicin, Streptomycin, Septrin and Ampicillin. The *Pseudomonas sp* was Susceptible to Tarivid by 100%, and resistant to Reflacin, Streptomycin, Septrin and Ampicillin by 100%. The statistical analysis revealed that Age, Occupation, Cause of wound and Educational status of the patient had significant effect on infection with *Pseudomonas aeruginosa* ($P < 0.05$). The presence of *Pseudomonas aeruginosa* in wounds could lead to the degeneration of such wounds, and thereby causing delay in the healing of the wounds.

Key words: Isolation, *Pseudomonas aeruginosa*, wounds, patients.

INTRODUCTON

Contamination of wound by bacteria is one of the most important cause of mortality and morbidity, novel nosocomial pathogens and antibiotics resistance leads to reviewing susceptibility patterns of several organisms isolated from wounds, one of which is *Pseudomonas aeruginosa*. (Mehta *et al.*, 2007). Most of the time, development of wound infections is largely dependent on two factors which are; impairment of host natural defence system and systemic dissemination of colonizing organism (Adebayo *et al.*, 2003). Wounds are majorly classified as accidental which can be deep or superficial, pathological which can be wound from diabetic foot (diabetes mellitus), ulcer, abscess, and post-operative wounds also known as surgical wounds (Bowler *et al.*, 2012). Other wounds can be

due to animal bites (snakes, dogs etc.). Whatsoever the nature of the wound may be, infection in wound is caused by attachment of microorganism to host cells where they proliferate, colonize and become better adapted to cause damage to the host tissues (Collier, 2003). So many variety of microorganisms have the ability to infect wounds and they range from bacteria to fungi and parasites. Constant widespread use of antibiotics has led to the emergence of resistant pathogens which has contributed immensely to mortality and morbidity. (Nwachukwu *et al.*, 2009). It has also been discovered that wound infections can be found world over, but the organism will usually vary with the geographical area, the normal flora resident on the skin and clothing at the site of wound (Anupurba *et al.*, 2010).

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In general, wounds can be said to be infected with bacterial cells if pus-like liquid emanates from it, even without culturing to determine positive result. Many wounds are usually colonized by bacteria even if they are not infected. Culturing of some wound samples may not yield pathogens, and this is because some of these organisms are fastidious in nature i.e. special nutrient required for their growth (Nwachukwu *et al.*, 2009). Infections in wound can be caused by many bacterial agents. Injury to the skin leads to coagulation and acute inflammatory response which is followed by exposure of inner skin tissues (subcutaneous) which will subsequently bring about loss of skin integrity and it will provide moist, warm nutrient and conducive environment for microbial proliferation or multiplication. Although there has been an introduction of a wide variety of antibiotics and antimicrobial agents with anti-*Pseudomonas* activity. There has been an incessant increase in the activity of *Pseudomonas aeruginosa* in wounds and it contributes to morbidity and mortality in hospitalized patients (Nwachukwu *et al.*, 2009). *Pseudomonas aeruginosa* is a gram negative rod shaped organism that has ability to resist antimicrobial agents and that is the main reason it can be found in wounds and nosocomial infections. It can colonize a wound rapidly and this makes it very difficult to deal with and it will usually make antimicrobials ineffective (Nagoba *et al.*, 2009).

MATERIALS AND METHODS

Sample collection: A total of 100 wound samples were collected using swab sticks from, both admitted patients and out-patients that attended the General Hospital, Minna. Questionnaire was administered to assess social demographic factors of patients before sampling. Following laboratory rules of sample collection, surface of wound was first cleaned with normal saline in order to exclude indigenous microbial population, and to reduce microbial load (Cheesbrough, 2006).

Isolation of *Pseudomonas aeruginosa* from infected wound samples.

Collected samples were collected using sterile peptone water and transported to the laboratory where a sterile wire loop was used to inoculate samples onto solidified cetrinide agar, which is selective for the isolation of *Pseudomonas* from infected samples. Plates were incubated for 24hrs at

37°C, after which plates were observed for cultural characteristics of *Pseudomonas aeruginosa* isolates (Cheesbrough, 2006)

Biochemical characterization of the isolates.

Suspected pure colonies were subjected to different biochemical tests after gram staining according to Cheesbrough (2006) for proper identification. The biochemical tests include catalase, coagulase, oxidase, motility, urase, indole production, methyl red, Voges-Proskauer, citrate utilization and sugar fermentation.

Antibiotics Susceptibility Testing

This test was carried out to determine the susceptibility profile of the isolates to some therapeutic agents used in hospitals. It was performed on Mueller-Hinton agar using the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards, by preparing the 0.5 McFarland's standard to standardize the turbidity of the isolates' suspension. The suspension of the *Pseudomonas* isolates were prepared from overnight incubated isolates on the Nutrient agar slants, which were later inoculated evenly on the surface of sterile Mueller-Hinton agar plates using sterile swab sticks, after which multi discs antibiotics were placed on the surface of inoculated plates and incubated at 37°C for 24 hours. Reading and measuring of zones of inhibition was done in millimeters. Isolates were ranked Susceptible or resistant by comparing the zones of inhibitions with the values that are recommended on the standard charts (CLSI, 2012).

Statistical Analysis

One-way Anova was used to determine the association between the social demographic factors and the occurrence of *Pseudomonas aeruginosa* in the wounds patients.

RESULTS

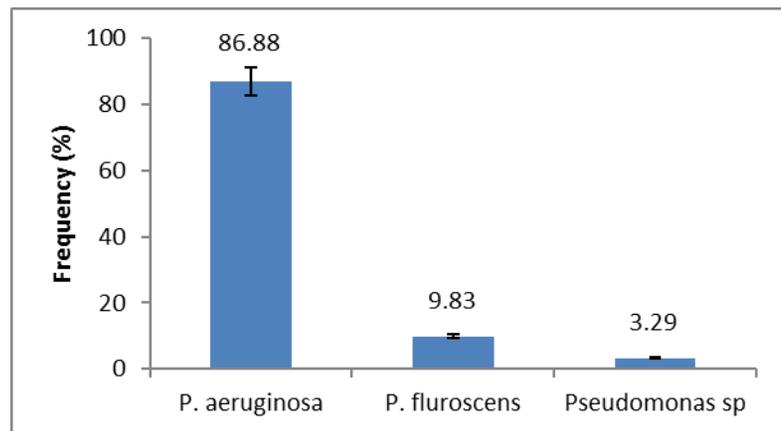


Figure I: Comparative prevalence of Species of Pseudomonas isolated. Different species of *Pseudomonas* were isolated from the wounds of the patients, where *P. aeruginosa* ranked highest with Fifty- three 53(86.88%) followed by *P. fluorescens* 6(9.83%) and *Pseudomonas sp.* 2(3.29%), as shown in the figure above.

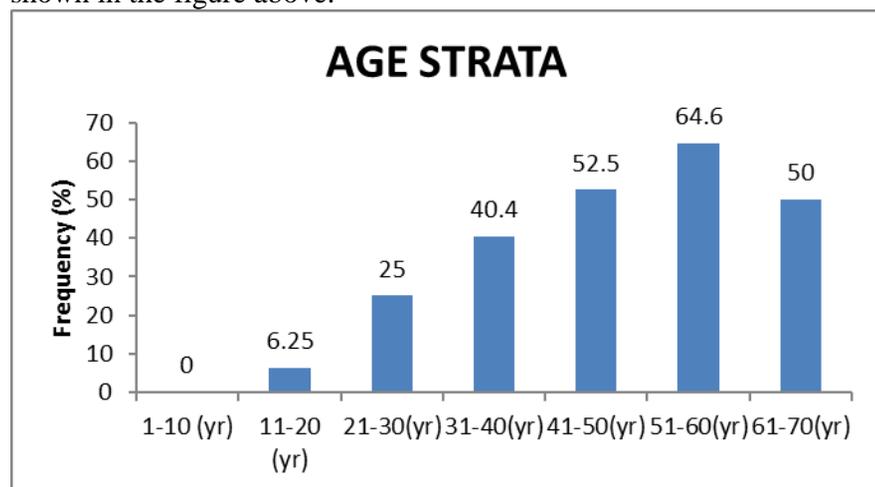


Figure II: Occurrence *P. aeruginosa* by age of the patients. Statistically significant at (P<0.05)

The distribution of the bacteria among various age groups of the patients in the figure above indicated that there is association between the age of the patients and the occurrence of the bacteria in their wounds. This implies that age is statistically significant at (P< 0.05).

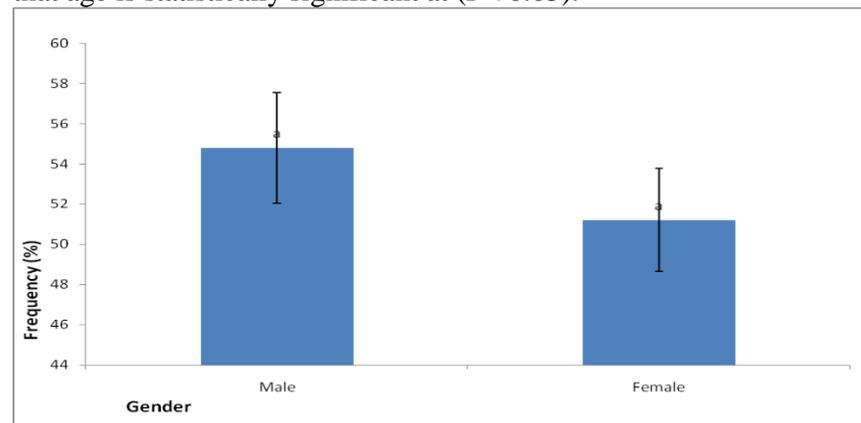


Figure III: Occurrence of *P. aeruginosa* by the gender of the patients. Gender is statistically not significant (P>0.05).

Following the distribution of *Pseudomonas aeruginosa* among male and female patients, no statistical significance was shown (P>0.05) as indicated in the figure above.

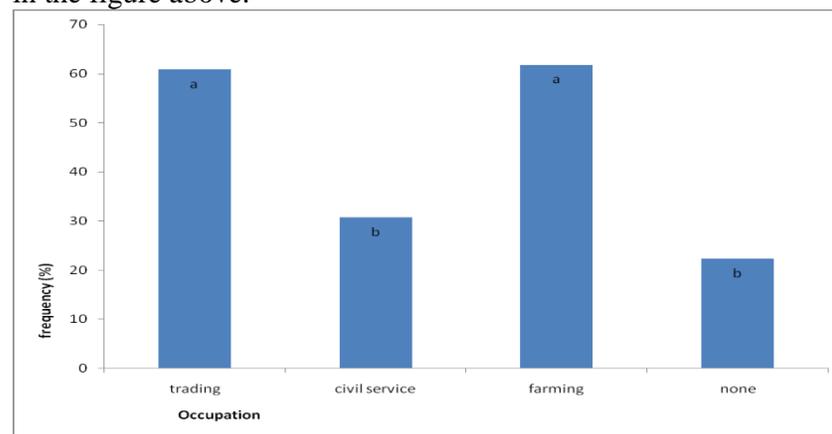


Figure IV: Occurrence of *P. aeruginosa* by the occupation of the patients.

Bars bearing different alphabets are statistically significant ($P < 0.05$).

The distribution among different occupational groups indicated that the occupation of the patients is statistically significant judging by the significant difference ($P < 0.05$), therefore, occupation is a determining factor for *Pseudomonas aeruginosa* to be present in a patient's wound.

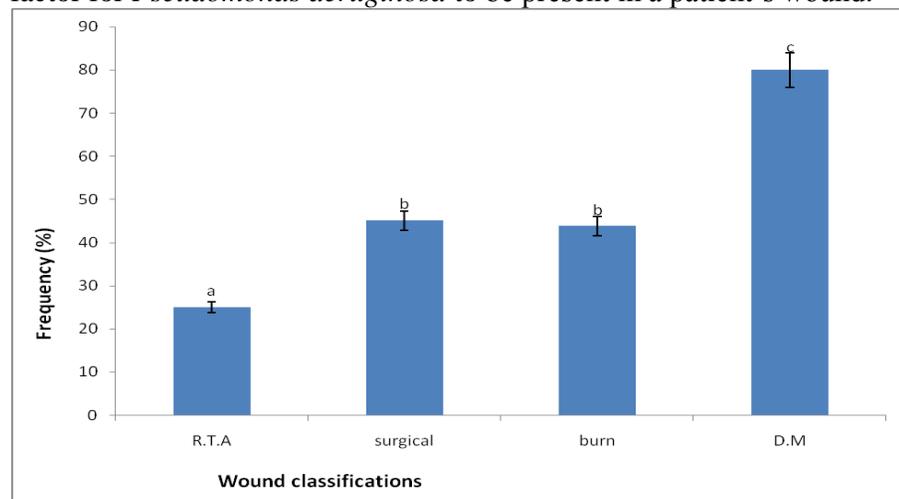


Figure V: Occurrence of *P. aeruginosa* by the cause of wound of patients.

Bars bearing different alphabets are statistically significant ($P < 0.05$).

The different causes of wound is statistically significant ($P < 0.05$), therefore the different causes of wound becomes significant to determine whether the wounds of the patients contain *Pseudomonas aeruginosa* or not.

KEY:

DM = Diabetes mellitus.

R.T.A= Road traffic accident

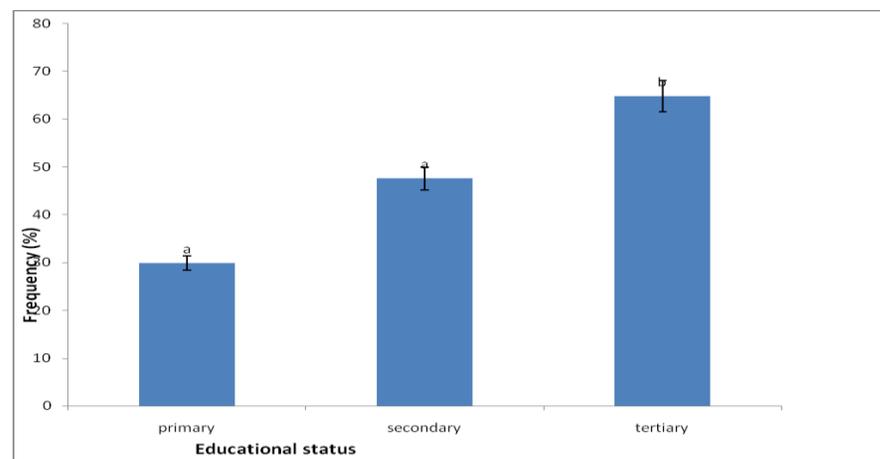


Figure VI: Occurrence of *P. aeruginosa* among patients of various educational backgrounds.

Bars bearing different alphabets of the Educational status are statistically significant ($P < 0.05$) as indicated in the graph above.

TABLE I: Percentage Susceptibility of the Isolates to Antibiotics.

Antibiotics Disc Potency(μ g)	P. aeruginosa n = 53	P. fluorescens n = 6	Pseudomonas sp. n = 2
OFX	10 86.8 (13.2)	33.3 (66.7)	100 (0)
PEF	10 56.6 (13.4)	16.7 (83.3)	0 (100)
CPX	10 94.3 (5.7)	83.3 (16.7)	50 (50)
AU	30 73.5 (26.5)	50 (16.7)	50 (50)
CN	10 20 (37.7)	0 (100)	50 (50)
S	30 0 (100)	0 (100)	0 (100)
CEP	10 37.7 (62.3)	33.3 (66.7)	50 (50)
NA	30 28.3 (71.7)	16.1 (71.7)	50 (50)
SXT	30 3.7 (96.3)	0 (100)	0 (100)
PN	30 0 (100)	0 (100)	0 (100)

Susceptibility to antibiotics is shown outside the parenthesis, while resistance is shown within the parenthesis. Analyzing the Susceptibility result, *P.aeruginosa* isolates showed high Susceptibility to Ciprofloxacin, Tarivid and Augmentin, and were 100% resistant to Streptomycin and

J. Baba, V.E. Ajaegbu, S.B. Mohammed, M. Abdullahi, O.A. Olutimayin, Y. Zakari Ampicillin. *P. fluorescens* isolates showed high Susceptibility to Ciprofloxacin only but were 100% resistant to Gentamycin, Streptomycin, Septrin and Ampicillin. *Pseudomonas* sp exhibited 100% Susceptibility to Tarivid and equally resisted Reflacin, Streptomycin, Septrin and Ampicillin by 100%.

KEY:

3167 OFX = Tarivid. PEF = Reflacin. CPX = Ciprofloxacin. AU = Augmentin. CN = Gentamycin. S = Streptomycin. CEP = Ceporex. NA = Nalidixic acid. SXT = Septrin. PN = ampicillin.

DISCUSSION

This study implicated *Pseudomonas aeruginosa* and other species of the bacteria as possible etiological agents in the cases of wound infections observed. These bacteria includes; *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas* sp. Among the isolated bacteria, *P. aeruginosa* had the highest prevalence of 86.88%, and this is because it is a gram-negative Bacteria which is highly associated with wound infections, this is supported by the report of Kehinde *et al.* (2004) and Anupurba *et al.* (2010), but in contrast to the study carried out by Opalekunde *et al.* (2014). The predominant nature of the organism might be due to its presence in virtually all environments and even hospitals, possession of virulent properties which aids adherence to host tissues, which agrees with the study carried out by Opalekunde *et al.* (2014) which states that high prevalence of *P. aeruginosa* may be due to endogenous source of infection, and Cruciani *et al.* (1998) who reported that strains of *P. aeruginosa* is constantly being isolated from medical equipment and biofilm surfaces.

Demographic data of positive cases of infection due to the bacteria were examined by age strata and it was shown that there is a statistical significant difference ($P < 0.05$), which agrees with a research report of Prvitts *et al.* (2001) which states that wound infection occurs more in adults than in children. Occupational factor was also assessed and it showed that occupation is a predetermining factor in the occurrence of wound infection due to *P. aeruginosa*, which means that it had statistical significance ($P < 0.05$). Occurrence of *P. aeruginosa* by the cause of wound was also analyzed and it showed a Statistical significance ($P < 0.05$), and this goes to show that wound colonization by the bacteria may depend on the type of wound. The result shows

that *P. aeruginosa* may not frequently colonize wounds due to road traffic accidents, because, they are fresh wounds, the Bacteria may be highly associated with surgical wounds due to the presence of the bacteria in hospital environments and surgical equipments. Prevalence in burn may be because, burn wounds are sometimes fresh and also because large area of the wounds are usually exposed, leading to high level of bacteria proliferation and lastly, highest prevalence was seen in wounds due to diabetes mellitus, because of difficulty in wound healing, the usual lengthy period of wound occurrence, which may cause the wounds to become infected during the process of treatment in the hospital and in other environments. This finding is supported by work done by Garba *et al.* (2014), where it was stated that exposed tissues in burn wounds are ideal for bacterial proliferation, and that *P. aeruginosa* is always isolated from surgical wounds. Educational status was also examined statistically and a significant difference was obtained ($P < 0.05$). Statistical analysis of gender did not show any significance ($P > 0.05$), this in contrast to the study carried out by Srinivas *et al.* (2001).

The susceptibility of *P. aeruginosa* isolates to antibiotics used differs. Out of the fifty-three (53) positive *P. aeruginosa* isolated, 46 (86.8%) were sensitive to Ofloxacin (tarivid) and Ciprofloxacin, 39 (73.5%) were sensitive to Augmentin, 30 (56.6%) were also sensitive to Reflacin and exhibited 100% resistance to Streptomycin and Ampicillin, which may be due to over use of this antibiotic, this study is supported by research findings of Garba *et al.* (2014) where it was stated that *P. aeruginosa* was susceptible to Ofloxacin and Augmentin, but in contrast, *P. aeruginosa* was sensitive to Ampicillin. This finding in this study is also in agreement with Opalekunde *et al.* (2014) which stated that *Pseudomonas* sp are resistant to Streptomycin and Ampicillin, it was also reported that *P. aeruginosa* was sensitive to Ciprofloxacin. The resistance shown to Streptomycin and Ampicillin may be due to usage over a long period of time, the inconsistency of some patients to the use of antibiotics and the rate in which they have been absorbed into the blood stream. This is in conformity with the findings of Mohammed *et al.* (2013), where it was stated that the over-use of antibiotics contributes to a patient developing resistance to a particular antibiotic. Sensitivity to Ofloxacin and Ciprofloxacin may be due to the fact that they are broad spectrum, less readily available to

patients, because they are rare and expensive, which means they may not have been miss-used as earlier reported by Vorland (2001), who stated that the success of Ciprofloxacin could be due to its broad spectrum activities which occurs in bacteria in both resting and replicative phases in order to destroy the deoxyribonucleic acid (DNA) and cause death of cell.

Conclusion and recommendations

Pseudomonas aeruginosa is the predominant organism isolated from wound samples screened in this study. This study was found to be in line with previous reports in which *P. aeruginosa* was found to be frequently isolated from infected wounds. The high resistance shown to some antibiotics can be due to inappropriate use of drugs. There is therefore a need to put in place effective surveillance measures in a bid to check the spread of infection due to the studied organism. *Pseudomonas aeruginosa* was discovered to be the most important cause of morbidity and mortality in patients whose wounds are infected. This study highly recommends the use of Ofloxacin, Ciprofloxacin and Augmentin for treatments of wound infections due to the study Bacteria. Efforts should be made by the government of the country and states to create awareness on hygiene practices and on proper use of antibiotics with emphasis on the inherent danger of self medication. Caregivers should make efforts to stay up to date on the susceptibility pattern of *P. aeruginosa* and other organisms predominant in wound infection and other cases of infection. Patients should be sensitized on the need to report wounds early enough to the Hospital to avoid wound degeneration.

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Conflict of Interest.

No conflict of interest.

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