Incidence and Susceptibility Pattern of Clinical Isolates of *Pseudomonas aeruginosa* from Selected Teaching Hospitals in Southwest Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author IAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SLO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Despite advances in sanitation facilities and the introduction of various antimicrobial agents with anti-pseudomonal activities, the life-threatening infections caused by *Pseudomonas aeruginosa* has continue to be hospital infections. This study was aimed to determining the incidence and susceptibility patterns of *P. aeruginosa* from some teaching hospitals in Southwest, Nigeria. Seventy-seven (77) isolates of *Pseudomonas* species were obtained from different clinical specimens from three (3) teaching hospitals in southwest, Nigeria. The isolates were re-identified by culturing on cetrimide agar plate and oxidase test was performed on the isolates. Information on the site of isolation, age and gender of the patient were obtained from request forms. *Pseudomonas aeruginosa* infections is mostly associated with the age range of 30-39 years in male patients and 10-19 years in female patients (P<0.05). The wound swab (29.87%) has the highest rate of *P. aeruginosa* infections closely followed by ear swab (22.08%). There was a statistical significant increase in the mean diameter of zone of inhibition of ciprofloxacin against *P. aeruginosa* when compared with other antibacterial agents (F-ratio = 18.798, P< 0.0001). However, *P. aeruginosa* was absolutely resistant to ceftazidime and augmentin.

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1. INTRODUCTION

Pseudomonas aeruginosa is a motile, non-fermenting, Gram-negative organism belonging to the family Pseudomonadaceae. [1] reported that it was observed that a blue-green discharge was frequently present and associated with infection in surgical wound dressings and the infectious organism has a rod-shaped and blue-green pigmented bacterium [1]. Thus, the ability of this organism to cause both severe acute and chronic infections were recognized [1].

Pseudomonas aeruginosa is an opportunistic pathogen that causes a wide range of acute and chronic infections in patients who are immune compromised or has other predisposing conditions. It can infect almost all tissues and it is a common cause of nosocomial infections [2]. Despite anti-pseudomonas activity being one of the pharmaceutical drug discoveries for several decades, it remains one of the most recalcitrant and difficult to treat organisms [1].

Generally, inadequate antimicrobial treatment defined as ineffective treatment of infection is an important factor in emergence of antibiotic resistant bacteria [3]. Factors that contribute to inadequate antimicrobial treatment of hospitalized patients include: the prior use of antibiotic, broad spectrum antibiotics, prolonged hospital stay and the presence of invasive medical devices. Other factors include the spread of resistant organisms through overcrowding and inadequate hospital infection control practices [3]. However, there are a number of clinical diseases associated with P. *aeruginosa* infection. *P. aeruginosa* is an opportunistic organism infecting; burn, CF, leukemic, transplant, neutropenic, long-term urinary catheters, and diabetic patients as well intravenous drug abusers [1].

The high prevalence of antimicrobial resistance observed among *P. aeruginosa* isolates underlines the strict consideration in antibiotics use at clinical settings [4]. One of the challenges in managing *P. aeruginosa* infections is an inherent resistance mechanism, referred to as intrinsic resistance. Its multiplicity of resistance mechanisms may render this microbe less amenable to control by antibiotic cycling [5].

P. aeruginosa is noted for its metabolic versatility and its exceptional ability to colonize a wide variety of environments and also for its intrinsic resistance to a wide variety of antimicrobial agents. The bacillus almost never causes infections in healthy individuals and often infects the immune compromised. Because of its virulence and the limited choices of effective antimicrobial agents, treatments of infections by *P. aeruginosa* are often difficult [5].

It is however important to perform antibiotic surveillance programs for appropriate empirical therapy and infection control practices. An increase in resistance to different anti-pseudomonal drugs particularly among hospital strains, has been reported world-wide and this has raised concern in therapeutic management of disease due to this organism [4]. This study therefore investigates the incidence and susceptibility patterns of isolates of *P. aeruginosa* obtained from different clinical samples.

2. MATERIALS AND METHODS

2.1 Sample Collection

Isolates of *Pseudomonas* species isolated from different samples were obtained from the routine bacteriological laboratories of Olabisi Onabanjo University Teaching Hospital (OOUTH) Sagamu, University College Hospital (UCH) Ibadan, and Federal Medical Centre (FMC) Abeokuta (all in Southwest, Nigeria). Also, information about the gender, age and different sites of isolation were obtained from the laboratory request form.

2.2 Identification of *Pseudomonas aeruginosa*

The identification of *P. aeruginosa* was achieved by the procedures of oxidase test and growth on cetrimide agar.

Cetrimide agar (Lab M, Lancashire, Uk) was purchased and agar plates were prepared according to manufacturer's instructions. All samples collected from different teaching hospitals were subcultured on the cetrimide agar plates and were incubated at 37°C for about 24hrs.

2.3 Oxidase Test

Few drops of oxidase reagent (Dalynn, Alberta, Canada) were poured on filter paper. An applicator stick was used to pick colonies of identified organisms (*P. aeruginosa*) and rubbed on the surface of the filter paper. A change in color was observed as positive reaction which shows a purple coloration of the rubbed organism.

2.4 Agar Disc Diffusion

The antimicrobial susceptibility test was performed on Mueller Hinton agar (Lab M, Lancashire, UK) using Kirby Bauer disk diffusion method. Mueller Hinton agar plates were prepared according to manufacturer's instructions and the 0.5 McFarland standard broth suspension of *P. aeruginosa* were flooded on it. A multidisc (Abtek, Liverpool, UK) containing different antibiotic at different concentration (in microgram) were carefully placed and the plates were incubated at 37°C for 24hrs, after which the diameter zone of inhibition of the drugs against the organism were read in millimeter [6].

Statistical analysis was achieved using ANOVA.

3. RESULTS AND DISCUSSION

A total of seventy-seven (77) isolates were collected from the three teaching hospitals within the southwestern region of Nigeria and they were subjected to various analysis. The prevalence of *Pseudomonas aeruginosa* according to age and gender of patients (Table 1) showed that *P. aeruginosa* infections was more associated with male patients of age range 30-39years and 10-19years in female patients (P<0.05).

In this study, the wound swab 23(29.87%) has the highest frequency of *P. aeruginosa* infections and it is closely followed by ear swab 17(22.08%) (Fig. 1). This refutes the report of [3] who showed that only 19.86% which is one-third of the infected wound was as a result of *P. aeruginosa*. The variation in this report may be due to the research environment, meanwhile this organism tends to be endemic in hospital environment by being easily transferred from object to object and they also tend to be resistant to common antiseptics, and often difficult to eradicate in the long term [7]. Peradventure, this does not agree with the work of [8]; who reported *S. aureus* as the predominant isolate in wound.

The identified *P. aeruginosa* isolates were tested against ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), cefixime (5µg), ofloxacin (5µg), augmentin (30µg), nitrofurantoin (300µg) and ciprofloxacin (5µg). From this study, the percentage antibacterial sensitivity and resistance of *P. aeruginosa* to commonly used antibacterial agents explained that ofloxacin and ciprofloxacin has the highest percentage of sensitivity against *P. aeruginosa* while *P. aeruginosa* was absolutely resistant to ceftazidine and augmentin simultaneously (Table 2).

Furthermore, the comparison of mean diameter zone of inhibition of various antibacterial agents against *P. aeruginosa* using agar disc diffusion method (Table 3) revealed a statistical significant increase in diameter zone of inhibition of ciprofloxacin against *P. aeruginosa* when compared with other antibacterial agents (F-ratio=18.798, P< 0.0001). Ofloxacin 47(61.04%) and ciprofloxacin 47(61.04%) has the highest percentage of sensitivity, closely followed by

Table 1. The prevalence of *P. aeruginosa* according to age and gender of patients

| Age range	Sex					
	Male		Female		Total	
	N	(%)	N	(%)	N	(%)
0-11months	1	(1.82)	0	(0.00)	1	(1.29)
1-9yrs	12	(21.82)	1	(4.55)	13	(16.88)
10-19yrs	8	(14.55)	8	(36.36)	16	(20.78)
20-29yrs	3	(5.45)	3	(13.64)	6	(7.79)
30-39yrs	14	(25.45)	0	(0.00)	14	(18.18)
40-49yrs	7	(12.73)	0	(0.00)	7	(9.10)
50-59yrs	3	(5.45)	6	(27.27)	9	(11.69)
60-69yrs	7	(12.73)	1	(4.55)	8	(10.39)
70&above	0	(0.00)	3	(23.64)	3	(3.90)
Total	55	(71.43)	22	(28.57)	77	(100)

N- Number tested, P<0.05

Table 2. Antibacterial activities of *P. aeruginosa* to commonly used antibacterial agents

| Antibacterial agents | N | *Pseudomonas aeruginosa* Isolates | | | |
| | | Sensitive | | Resistant | |
		n	(%)	n	(%)
CAZ	77	0	(0.0)	77	(100)
CRX	77	2	(2.60)	75	(97.40)
GEN	77	45	(58.44)	32	(41.56)
CXM	77	2	(2.60)	75	(97.40)
OFL	77	47	(61.04)	30	(38.96)
AUG	77	0	(0.0)	77	(100)
NIT	77	8	(10.39)	69	(89.61)
CPR	77	47	(61.04)	30	(38.96)

N- Number tested, n- number sensitive or resistant

CAZ – Ceftazidime (30µg), CRX – Cefuroxime (30µg), GEN – Gentamicin (10µg), CXM – Cefixime (5µg),OFL – Ofloxacin (5µg), AUG – Augmentin (30µg), NIT – Nitrofurantoin (300µg), CPR – Ciprofloxacin (5µg)
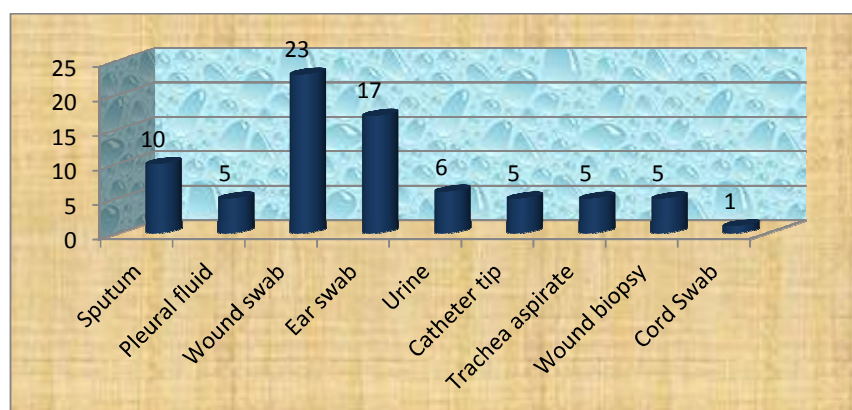
Fig. 1. Sample site of *Pseudomonas aeruginosa*

Table 3. Comparism of mean diameter zone of inhibition of various antibacterial agents against *P. aeruginosa* using agar disc diffusion technique

Various anti-bacteria Agents(unit)	N	Diameter zone of inhibition (mm) against *Pseudomonas aeruginosa* Mean ± SD
CAZ (30ug)	77	0.000 ± 0.000
CRX (30 ug)	77	14.000 ± 6.083
GEN (10 ug)	77	15.417 ± 2.805
CXM (5 ug)	77	17.800 ± 6.261
OFL (5 ug)	77	17.837 ± 3.804
AUG (30 ug)	77	0.000 ± 0.000
NIT (300 ug)	77	19.750 ± 6.151
CPR (5 ug)	77	23.480 ± 6.958
F-Ratio		18.798
P-Value		< 0.0001

N- Number tested, CAZ – Ceftazidime, CRX – Cefuroxime, GEN – Gentamicin, CXM – Cefixime, OFL – Ofloxacin, AUG – Augmentin, NIT – Nitrofurantoin, CPR – Ciprofloxacin

gentamicin 45(58.44%). However, this result disagree with studies of [9] in Nasiriyah, Iraq, where the results of the study demonstrated a high resistance to Ciprofloxacin, ofloxacin, cefepime, gentamicin, tobramycin, norfloxacin, levofloxacin and amikacin. The difference may be attributed to exposure to several antibiotics.

Moreover, all the isolates of *P. aeruginosa* were absolutely resistant to ceftazidine and augmentin. The general resistance of *P. aeruginosa* to augmentin and ceftazidine might be due to the pressure of prolonged usage and regular abuse in our society. This is not unconnected with the abuse of amoxicillin by the populace since antibiotics are still sold across the counter in some pharmaceutical and patent medicine stores in Nigeria [3]. However, the low level of resistance shown by the *P. aeruginosa* isolates to ciprofloxacin, ofloxacin (third generation floroquinolones) and gentamicin indicates that these three drugs are still effective in this region compared to others [3].

4. CONCLUSION

This study showed that *P. aeruginosa* is the major organism found in infected wound site and the males are more susceptible than females. The bacteria isolates were generally resistant to commonly used antibiotics but ciprofloxacin and ofloxacin were effective against the isolates. Hence, ciprofloxacin remains one of the most important antibiotics in the treatment of *P. aeruginosa* infections as it is both the most effective fluoroquinolone and one of the most widely used antibiotics against this bacterium.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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