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STUDIES OF ANTIBIOTICS RESISTANCE OF *PSEUDOMONAS AERUGINOSA* FROM CLINICAL AND ENVIRONMENTAL (WATER AND SOIL) SAMPLES.

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ABSTRACT

This study was carried out to investigate the antibiotic resistance pattern of some *Pseudomonas aeruginosa* isolates from clinical and environmental (water and soil) samples. The isolates from the environmental samples were identified using the standard cultural, morphological and biochemical characteristics. A total of 47 bacteria were isolated, 37 were Gram negative and out of these, 4 were *Pseudomonas aeruginosa*, one isolate was from water and three from soil samples. Four clinical isolates were also used. *P. aeruginosa* was resistance to Nalixicidic acid, nitrofurantcin, cotrimoxazole, amoxicillin, tetracycline, augmentin and ceftazidime while sensitive to gentamicin and ofloxacin. The zone of inhibition of *P. aeruginosa* from blood sample was highest with ofloxacin (30 mm) followed by sputum and urine samples of 29m each. For the environmental samples, *P. aeruginosa* from water and from one of the soil samples had the highest zones of inhibition of 30mm with ofloxacin. There is a need to study the antibiotic resistant pattern so as to be able to counteract emerging resistant patterns of *P. aeruginosa* from different sources.

KEYWORDS: *Pseudomonas aeruginosa*, resistance, antibiotics, environmental, clinical isolates.

1. INTRODUCTION

Pseudomonas aeruginosa, an increasingly prevalent opportunistic human pathogen, is the most common Gram-negative bacterium found in nosocomial infections. *P. aeruginosa* is responsible for 16% of nosocomial pneumonia cases[1], 12% of hospital-acquired urinary tract infections[2], 8% of surgical wound infections[3], and 10% of bloodstream infections.[4] It is the third most common pathogen associated with hospital acquired catheter- associated urinary tract infections (UTIs).[5] *Pseudomonas aeruginosa* is an important nosocomial pathogen, especially in individuals with neutropenia and those who are immuno-compromised.[1.6.7] The emergence of antibiotic resistant micro-organisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* is increasing rapidly around the globe creating a serious threat; many of the pathogens that cause nosocomial infection have a high level of resistance to antibiotic treatment.[8] Infections from drug resistant *Escherichia coli*, *Staphylococcus* aureus, and *Pseudomonas aeruginosa* are becoming common.[9] However, there is mounting evidence that these bacteria may be responsible for primary infections as a result of increased use of medical in dwelling plastic devices and compromised or immunodepressed patients.[5,10]

P. aeruginosa is considered the most challenging pathogen due to its resistance rate which is high to antimicrobial agents.[11,12] It flourishes in different ecological environments including soils, rivers, wastewater, plant, animal and human.[13] Environments containing antibiotic residues exert selection pressure and contribute to the appearance of resistant bacteria, many studies have focused on antibiotic resistant bacteria from various ecosystems.[14,15,16]

This study was carried out to determine the antibiotic susceptibility pattern of *Pseudomonas aeruginosa* from clinical, soil and water samples.

2. MATERIALS AND METHODS

2.1. Collection of samples

2.1.1.Water sample collection

The water samples were collected from three different rivers around Afe Babalola University, Ado- Ekiti using sterile plastic containers.

2..1.2. Soil sample collection

Soil samples were collected from soils around the rivers and a mechanic workshop in sterile polythene bags.

2.1.3. Clinical sample collection

Pure cultures of *Pseudomonas aeruginosa* isolated from different samples were collected from Ekiti State University Teaching Hospital, Ado Ekiti.

2.2. Preparation of culture media

The culture media were prepared according to manufacturer specifications and sterilized for 15 minutes at 121^{o}C.

2.2.1. Serial dilution

Serial dilution was carried out on both the water and soil samples. One gram of the soil sample was weighed and added into 10ml of sterile distilled water, after which serial dilution was carried out. One ml each from 10^{-2}, 10^{-3}, 10^{-4} were dispensed each in sterile Petri dishes containing Pseudomonas agar. Each plate was swirled for proper spreading (Pour plate method). The plates were allowed to dry and incubated at 37^{o}C for 24-48 hours. The same technique was used for the water samples using 1ml each for the different water samples.

2.3 Isolation of microorganisms

The isolation of the bacteria was carried out using Pseudomonas agar. Sub culturing of the bacterial isolates were done by streaking the bacteria colonies on Nutrient agar plates and incubated at 37^{o}C for 24 hours. After obtaining pure cultures, they were put on nutrient agar slants and kept in the refrigerator for further use.

2.4. Antibiotic susceptibility test

All the isolates organisms were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. This was carried out by making an even spread of the pure isolates on prepared Mueller-Hinton agar using sterile swab sticks and aseptically placing the antibiotics discs using sterile forceps. The plates were incubated aerobically at 37˚C for 24 hours after which the zones of inhibition were measured and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS). Antibiotics used were; NIT (nitrofurantcin); COT (co-trimoxazole); AMX (amoxycillin); TET (tetracycline); AUG (augmentine), CAZ (ceftazidime); OFL (ofloxacin); GEN (gentamicin); NAL (nalixicidic acid) of standard strengths.[17]

RESULTS

Three water samples were collected from three different rivers around Afe Babalola University, Ado- Ekiti while four soil samples were collected around the river banks and a mechanic workshop. Four clinical isolates of *Pseudomonas aeruginosa* were collected from Ekiti State University Teaching Hospital Ado- Ekiti. A total of forty seven organisms were isolated from both the soil and water samples, using Pseudomonas agar, out of which ten organisms were Gram positive, so further biochemical test were not carried out on these isolates. The remaining thirty seven isolates were Gram negative, and only four of these were *Pseudomonas aeruginosa* based on their shape and the biochemical tests carried out on them. Out of the water samples, only one organism was *Pseudomonas aeruginosa*, while three were from the soil samples. All the four organisms produced distinct greenish pigment. They were Gram negative and rod shaped. They were catalase and oxidase positive. On Maconkey agar, the isolates grew but did not turn pink showing that they were non lactose fermenters, all were indole positive and motile (result not shown).

Table 1 shows the antibiotic susceptibility test for the clinical isolates of *Pseudomonas aeruginosa*. They all showed resistance to some of the antibiotics such as nitrofrurantcin, cotrimoxazole, amoxycillin, tetracycline, augmentin, ceftazidime, nalixidic acid, but were susceptible to gentamicin and ofloxacin. OFL(ofloxacin) was more effective on the samples than gentamicin. From *P. aeruginosa* isolated from blood, the zone of inhibition exhibited by ofloxacin was 30mm which was the highest. The zone of inhibition exhibited by ofloxacin on *P. aeruginosa* from sputum and urine were 29mm each. For gentamicin, the isolates from urine and wound swab had inhibition zones of 19.0mm each and from sputum, 17.0mm.

Table 2 shows the antibiotic susceptibility test of samples from water W1 and soil S1, S2, S3. They were all resistant to nitrofurantcin, co-timoxazole, amoxycillin, tetracycline, augmentin, ceftazidime, nalixicidic acid and susceptible to gentamicin and ofloxacin. Ofloxacin showed the highest activity on *P. aeruginosa* from water sample -W1 and soil S3 with zones of inhibition of 30mm each. The other isolates had zones of inhibition of 25mm each. The highest zone of inhibition for gentamicin was observed on *P. aeruginosa* from soil sample – S3 which was 21mm while the least was from *P. aeruginosa* from water sample – Wl with zone of inhibition of 13mm.

Table 3 shows the comparative result of antibiotics susceptibility for both the environmental and clinical samples. They are resistant to antibiotics such as co-trimoxazole, amoxycillin, tetracycline, augmentine, ceftazidime, nalixidic acid, nitrofurantcin and were susceptible to gentamicin and oflaxacin.

Table 1: Antibiotic susceptibility test for *Psedomonas aeruginosa* from clinical isolates

Antibiotics	Samples/ zone of inhibition (mm)			
	Sputum	urine	Wound swab	Blood
NIT	-		-	-
COT	-	-	-	-
AMX	-	-	-	-
TET	-	-	-	-
AUG	-	-	-	-
OFL	29	29	28	30
GEN	17	19	19	18
NAL	-	-	-	-
CAZ	-	-	-	-

Key: - = No zone of inhibition Diameter of the disc= 2mm NIT= Nitrofurantcin COT= Co-trimoxazole AMX= Amoxycillin TET= Tetracycline AUG= Augmentin CAZ= Ceftazidime OFL= Ofloxacin GEN= Gentamicin NAL= Nalixicidic acid

Table 2: Antibiotic susceptibility test of isolates from soil and water samples.

ANTIBIOTICS	Samples/zone of inhibition(mm)			
	W1	S1	S2	S3
OFL	30	25	30	25
GEN	13	20	21	19
NAL	-	-	-	-
AUG	-	-	-	-
COT	-	-	-	-
AMX	-	-	-	-
TET	-	-	-	-
NIT	-	-	-	-
CAZ	-	-	-	-

Key:W1- Isolate from water sample, S1, S2, S3- isolates from soil samples. - = No zone of inhibition Diameter of the disc= 2mm NIT= Nitrofurantcin COT= Co-trimoxazole AMX= Amoxycillin TET= Tetracycline AUG= Augmentin CAZ= Ceftazidime OFL= Ofloxacin GEN= Gentamicin NAL= Nalixicidic acid.

Table 3: Result of antibiotics susceptibility test for both the environmental and clinical sample.

ANTIBIOTICS	SOIL AND WATER SAMPLES				CLINICAL SAMPLES			
	W1	S1	S2	S3	WOUND SWAB	BLOOD	URINE	SPUTUM
OFL	S	S	S	S	S	S	S	S
NIT	R	R	R	R	R	R	R	R
COT	R	R	R	R	R	R	R	R
AMX	R	R	R	R	R	R	R	R
TET	R	R	R	R	R	R	R	R
AUG	R	R	R	R	R	R	R	R
GEN	S	S	S	S	S	S	S	S
NAL	R	R	R	R	R	R	R	R
CAZ	R	R	R	R	R	R	R	R

Key:W1- Isolate from water sample, S1, S2, S3- isolates from soil samples. NIT= Nitrofurantcin COT= Co-trimoxazole AMX= Amoxycillin TET= Tetracycline AUG= Augmentin CAZ= Ceftazidime OFL= Ofloxacin GEN= Gentamicin NAL= Nalixicidic acid.

DISCUSSION

Pseudomonas spp. is the main cause of nosocomial infections causing morbidity and mortality as these infections are difficult to eradicate. There is a global emergence of multidrug resistant strains of *Pseudomonas*. The transmission of infection during patient remedy in hospital can occur by direct contact with surfaces.[18] Regarding the antibiotics disc used, it was found that *P. aeruginosa* was resistant to erythromycin, tetracycline, amoxicillin, nitrofurantcin, co-trimoxazole, nalixicidic acid, augmentin, ceftazidime and susceptible to gentamicin and ofloxacin.

It was reported in the study carried out by[19] on the prevalence and resistant pattern of *Pseudomonas aeruginosa* against various antibiotics, that the organism showed resistance against ofloxacin and ciprofloxacin and their resistant rates were between 70-98% while the resistant patterns against gentamycin, trobramycin, ceftazidim, amikacin, were observed to be less as compared to other drugs in their study. Their result was not similar to those obtained in this study because *Pseudomonas aeruginosa* was susceptible to ofloxacin and gentamicin. A report on the study carried out on contaminated soils from refuse dumps showed that many *P. aeruginosa* isolates were susceptible to both gentamicin and colistin.[20] This result is similar to those reported in this study regarding getamicin, also, the result by[21] was similar to the one obtained in this study which showed that *P. aeruginosa* was sensitive to getamicin and resistant to ceftazidime. It was reported by[22] that *Pseudomonas aeruginosa* from water and clinical samples were resistant to amoxycillin, tetracycline, genatmicin and nalixicidic acid, all in agreement with our work except for gentamicin. Other *Pseudomonas aeruginosa* from soil and water sample from their study was sensitive to gentamicin.

A survey conducted in two local hospitals by[23], found that the rate of resistance to ciprofloxacin was high, such that it presented activity against only 49.7% of all of the strains of *P. aeruginosa* and the susceptibility to aminoglycosides was 59.4% for amikacin and 48.6% for gentamicin. These results are also partly similar with those reported in this present study, just that ciprofloxacin and amikacin was not used. Aminoglycosides such as gentamicin can be used to treat *P. aeruginosa* infection, as reported by.[24] The best association of antimicrobial agents for treating infections due to *P. aeruginosa* is still a matter of controversy. The evolution of multi-resistant *P. aeruginosa* and its mechanisms of antibiotic resistance have been examined, and their primary mechanisms include reduced cell permeability, efflux pumps, changes in the target enzymes and inactivation of the antibiotics.[25,26]

CONCLUSION

Pseudomonas aeruginosa clearly shows that it is one of the most challenging pathogenic bacterium. There is the need for clinicians to implement prophylactic measures that are aimed at reducing not only nosocomial infections but also infections that can be caused by *P. aeruginosa* isolated from the environments. More work need to be carried out to know the resistance pattern and β lactamase production of the organism.

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