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Antibiotic Susceptibility Patterns of *Pseudomonas* spp. Isolated from Soil Samples

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Abstract
Antimicrobial resistance is one of the major problems facing the health sector. This has to do with many factors including changes in metabolic pathways and transfer of virulence genes among bacterial species among others. Therefore, this study aimed at testing the antibiotic susceptibility patterns of Pseudomonas spp. isolated from soil samples using the mastering-S (M13 and M14 disc). In this study, the susceptibility of 25 pseudomonas isolated from soil environment were tested using the mastering-S (13 and 14 disc). Overnight KB cultures were used to inoculate KB* plates using the spread plate technique before placing the antibiotic disc on the surface of the agar and incubated at 20°C for 48hours. Antibiotic susceptibility was recorded based on the diameter of zone of inhibition. A diameter of 8mm and above was recorded as positive (susceptible) and a value of less than 8mm as negative (resistance). The results show that, all 25 isolates (100%) tested resistant to at least eight (8) antibiotics, while all the strains were susceptible to 10 µg Gentamicin (GM). Similarly, at least 8% were susceptible to 25 µg Chloramphenicol (C), 96% are susceptible to 10 µg Streptomycin (S) and about 92%, 40% and 48% of the isolates were found to be susceptible to 25 µg Tetracycline (T), 25 µg Colistin Sulphate (CO) and 200 µg Sulphatriad (ST) respectively. Therefore, the study concludes that different species of pseudomonad may respond to antibiotic differently and this should be considered especially when selecting drug of choice in medical settings.*
***Key words**: Antibiotic, Mutation, Pseudomonas, Resistance and Susceptibility*

INTRODUCTION

Pseudomonas are group of Gram-negative aerobic rods that are actively motile and widely distributed in nature, they occur as free living organisms in soil, water and in decomposing organic matter. Members of the genus have the ability to utilized a great variety of compounds for energy production, they are mostly saprophytes or commensals but a few are pathogenic to plant and man, they are classified based on rRNA/DNA homology and common culture characteristics (Amadi *et al.*, 2018). In addition to these features, *Pseudomonas* spp. produces a wide variety of extracellular products which a number of functions linked to extracellular polymeric substances including proteins, biosurfactants, polysaccharides, enzymes, extracellular DNA etc, that are mainly used in attachment processes, virulence and biofilm formation (van Delden, 2004).

The biosurfactants produced by *pseudomonas* spp. increase the rate of solubilization of many hydrocarbons from non-aqueous phase liquids making them available for microbial degradation. Therefore, having an advantage to mineralize numerous organic compounds (aromatic hydrocarbons, chloro- and nitro-organic compounds, pesticides, herbicides) and play an important role in the bioremediationand detoxification of contaminated environments. *Pseudomonas* spp. has the ability to adapt and grow under limited O_2. Thus, serving as an important clinical feature, because most of the antimicrobial functions are reduced in anoxic condition (Line *et al.*, 2014), which may result to acquiring of numerous substance that can result to mutation, leading to resistance. Their ability to thrive under anoxic condition can also serve as an explanation to their limited antimicrobial susceptibility in patients with cystic fibrosis. (EARS-Net., 2013).

One of the most significant problem raised by researchers in connection with *pseudomonas* is their antibiotic resistance problem (Ghane and Azimi, 2014), For example, CDC (2013) reports that up to 6000 (13%) out of 51,000 health care infections caused by *P. aeruginosa* are multidrug-resistant cases. other reports, shows that *Pseudomonas* spp. shows a limited susceptibility to many antimicrobial agents.

Some species use the natural (intrinsic) resistance ability together which other mechanism such as low outer membrane permeability, ß-lactamases synthesis, and the efflux systems (Luczkiewicz *et al.*, 2015) to resist action by antibiotics. Moreover, due to the well-known plasticity of the members 'genome, many *Pseudomonas* spp. is suspected to have the ability to acquire almost all known antimicrobial-resistance mechanisms (Livermore, 2002).

Therefore, most infections caused by *Pseudomonas* are associated with multi-drug resistance with limited treatment options.

The afore mentioned reasons and other reasons not mentioned alert the need for continual research on more effective antibiotics that will solve the problem of resistance species and more susceptibility researches to ascertain the effect of the already known antibiotics.

Moreover, many researchers focused on the resistance and susceptibility of *Pseudomonas* spp. isolated in hospital settings. Only few studies have been reported on other environmental settings such as water and soil settings. Therefore, this study aims at testing the antibiotic resistance pattern of different pseudomonads species isolated from soil samples. This study will help to evaluate species containing antibiotics resistance potential for further studies.

MATERIALS AND METHODS

Pseudomonas spp. was isolated from Dundee Botanic garden soil using the grid sampling techniques. Soil samples were obtained during five visits to the garden and collected close to the following trees: Cornelian Cherry (*Cornus Mas*),Hance (*HemipteleaDavidii*), Moroccan Cypress (*Cupressus Atlantica*),Scots Pine (*PinusSylvestris*)and White Willow (*Salix Alba*).

To isolate *Pseudomonas* spp. from the soil samples, serial dilutions were performed and aliquots were inoculated on *Pseudomonas* selection agar (PSA+CFC) plates using the spread-plate method. Discret colonies were re-streaked on PSA+CFC to make an exenic culture. A total of 25 bacterial strains were isolated for further studies from the Five (5) plates each of the five soil samples.

Specie Identification

Out of the twenty five (25) isolates, Sixteen (16) isolates were selected and characterized using the Analytical profiling index (API 20e) according to manufacturer's instruction to assess their metabolic profile and to classify them to specie level.

Antibiotics Susceptibility

The 25 strains were tested for susceptibility to antibiotics using the MASTERING-STM antibiotic discs M13 and M14 (MASTERING-STM refers to an antibiotic ring device used to measure the sensitivity of more than six antibiotics simultaneously). Overnight KB* cultures were used to inoculate KB* plates using the spread plate technique before placing an antibiotic disc on the surface of the agar and incubating it at 20°C for 48 hours.

Antibiotic susceptibility was recorded based on the diameter of the zone of inhibition. A diameter of 8 mm and above was recorded as positive (Susceptible) and a value of less than 8mm as negative (not susceptible).

Tetracycline resistance

To further confirm and study the susceptibility of strains to antibiotics, strains were re-assessed for resistance to tetracycline using a modified version of the protocol reported by Kelch and Lee (1978). Tetracycline plates were initially prepared using LB media supplemented with 100 µg/mL of kanamycin. 10 µL of overnight KB* culture was drop-inoculatedonto plates before incubation at 20°C for 48 hours. A positive result was recorded if bacterial growth was observed and plates without growth were recorded as negative.

RESULTS

Twenty five (25) strains were selected from the *Pseudomonas* spp. selection agar plates. The strains were confirmed as *Pseudomonas* using the API 20e kit. This was done by comparing the strains metabolic profile with the API data base while out of the 16 strains that were identified to specie level, 12 were confirmed as pseudomonads (Table 3), one as *Aeromonas salmonicida* with the API 20e database not able to identify the remaining three.

The mastering-S disc asses the organism susceptibility based on the following antibiotics. For M13 the antibiotics are 25 µg Chloramphenicol (C), 5 µg Erythromycin (E), 10 µg Fusidic Acid (FC), 5 µg Oxacillin (OX), 5 µg Novobiocin (NO), 1-unit Penicillin G (PG), 10 µg Streptomycin (S) and 25 µg Tetracycline (T). while the M14 has the following antibiotics: 10 µg Ampicillin (AP), 5 µg Cephalothin (KF), 25 µg ColistinSulphate (CO), 10 µg Gentamicin (GM), 200 µg Sulphatriad (ST), 25 µg Cotrimoxazole (TS). Table 1 and 2 below, shows the individual susceptibility of all the antibiotics against the 25 isolates.

Table 1: The Value of the Zones of inhibition of *Pseudomonads* spp. to Antibiotics on the M13 Antibiotic Disc

	Diameter of the zone of inhibition for M13 antibiotics (mm)							
Strains	Chloromphenicol (C)	Erythromycin (E)	Fusidic Acid (FC)	Oxacillin (OX)	Novobiocin (NO	Penicilin G (PG)	Streptomycin (S)	Tetracyclin (T)
1	6	6	6	6	6	6	19	10
2	6	6	6	6	6	6	9	9
3	6	6	6	6	6	6	17	9
4	6	6	6	6	6	6	12	8
5	6	6	6	6	6	6	18	10
6	6	6	6	6	6	6	19	11
7	6	6	6	6	6	6	10	9
8	6	6	6	6	6	6	15	9
9	6	6	6	6	6	6	13	10
10	6	6	6	6	6	6	11	9
11	6	6	6	6	6	6	10	8
12	6	6	6	6	6	6	18	10
13	6	6	6	6	6	6	18	10
14	18	6	6	6	6	6	16	10
15	6	6	6	6	6	6	17	9
16	6	6	6	6	6	6	15	9
17	8	6	6	6	6	6	7	9
18	6	6	6	6	6	6	15	9
19	6	6	6	6	6	6	10	9
20	6	6	6	6	6	6	10	9
21	6	6	6	6	6	6	16	10
22	15	6	6	6	6	6	21	15
23	6	6	6	6	6	6	16	9
24	6	6	6	6	6	6	15	9
25	6	6	6	6	6	6	16	9

Table 2: The Values of the Zone of Inhibition of *Pseudomonads* spp. to Antibiotics on the M14 Antibiotic Disc

	Diameter of the zone of inhibition for M14 antibiotics (mm)					
Strains	Ampicillin (AP)	Cephalothin (KF)	Colistin Sulphate (CO)	Gentamicin (GM)	Sulphatriad (ST)	Cotrimoxazole (TS)
1	6	6	15	17	6	6
2	6	6	12	16	6	6
3	6	6	6	14	8	6
4	6	6	13	16	10	6
5	6	6	6	20	15	6
6	6	6	6	19	15	6
7	6	6	6	16	19	6
8	6	6	6	18	6	6
9	6	6	13	15	6	6
10	6	6	6	14	20	6
11	6	6	6	16	20	6
12	6	6	6	21	19	6
13	6	6	6	21	6	6
14	6	6	13	15	6	6
15	6	6	6	17	16	6
16	6	6	12	14	14	6
17	6	6	14	15	6	6
18	6	6	6	21	6	6
19	6	6	6	16	6	6
20	6	6	13	15	6	6
21	6	6	6	19	6	6
22	6	6	15	20	6	6
23	6	6	13	17	14	6
24	6	6	6	18	14	6
25	6	6	6	18	14	6

A total of 14 different antibiotics were tested to ascertain the antibiogram of the 25 *Pseudomonas* spp. isolates. Similarly, any isolates found to possess a diameter of zone of inhibition of ≤8 is regarded as resistant while a diameter of >8 is regarded as susceptible. Using this rule, all 25 isolates (100%) tested resistant to 5 µg Erythromycin (E), 10 µg Fusidic Acid (FC), 5 µg Oxacillin (OX), 5 µg Novobiocin (NO), 1-unit Penicillin G (PG), 10 µg Ampicillin (AP), 5 µg Cephalothin (KF), 25 µg Cotrimoxazole (TS). While all the strains were susceptible to 10 µg Gentamicin (GM). At least 8% were susceptible to 25 µg Chloramphenicol (C), 96% are susceptible to 10 µg Streptomycin (S) and about 92%, 40% and 48% of the isolates were found to be susceptible to 25 µg Tetracycline (T), 25 µg ColistinSulphate (CO) and 200 µg Sulphatriad (ST) respectively.

Further testing of the antibiotic resistance to tetracycline using 100 µg/ml concentration result in no growth of the organisms

Table 3: Strain Identification by the Analytical Profile Index (API e20) and 16S rDNA sequencing

Strain	API ID	API Sig. specie
Strain 1	2027046	*P. fluorescens/putida*
Strain 2	2026044	*P. fluorescens/putida*
Strain 3	2136046	*P. aeruginosa*
Strain 4	2226046	*P. fluorescens/putida*
Strain 5	2326046	*P. aeruginosa*
Strain 6	2026006	*P. fluorescens/putida*
Strain 7	2026004	*P. fluorescens/putida*
Strain 8	2036046	*P. aeruginosa*
Strain 9	3027056	*P. luteola*
Strain 12	3127004	Unacceptable/*Bibersteiniatrehalosi*
Strain 15	2127006	*P. fluorescens/putida*
Strain 17	2126046	*P. fluorescens/putida*
Strain 19	4127004	Unacceptable/*Burkholderiacepacia*
Strain 21	2125006	*P. fluorescens/putida*
Strain 23	2122105	*Aeromonassalmonicida*ssp
Strain 25	7325317	unacceptable

Key:
API ID = Analytical profile Index number.
API Sig. specie = API bacterial strain as identified by the API e20 database.

DISCUSSION

Environment represents a conducive environment where virulence genes including resistance to some certain antibiotics may be shared among microorganisms. In this research, twenty-five pseudomonas isolates were isolated from the rhizosphere zone of the plants. Although some of the isolates where identified as pseudomonads using the API e20, the result is purely using metabolic profile which may have some shortcomings especially when differentiating members of a genera to specie level. This is a problem even when 16S rDNA is use for the identification of pseudomonads to species level (Bossis *et al.*, 2000; Moore *et al.*, 1996). This corresponds to a study by Yamamoto *et al.*, (2000) that indicates that *Pseudomonas* spp. identification could not be resolved using 16S rDNA sequencing alone. Similarly, a review by Janda and Abbott, (2002), shows that all methods used to classify bacteria to species level have limitations because no method can provide results that are 100% reliable.

The susceptibility characteristics of an organism represent one of it is important behavior. This is because, it will guide researchers on so many important decisions including strain selections for genetic modification, drug development and in sensitivity studies where drug of choice is needed for administration.

Although there are very few documented researches using the antibiotics used in these research, the result of the research shows that all the 25 isolates tested are to 5 µg Erythromycin (E), 10 µg Fusidic Acid (FC), 5 µg Oxacillin (OX), 5 µg Novobiocin (NO), 1-unit Penicillin G (PG), 10 µg Ampicillin (AP), 5 µg Cephalothin (KF), 25 µg Cotrimoxazole (TS). This clearly shows that none of these drugs can be used in treating diseases resulting from any of the 25 isolates or as a selection maker for genetic modification. This resistance may be due to the concentration of the antibiotics on the disc, this is because antimicrobial concentration is among the key factors that influence susceptibility of an antibiotic (Li *et al.*, 2017) as this assertion where confirmed when 100 µg/ml of Tetracycline was used to further screen the isolates for sensitivity.

In contrast, all the strains were susceptible to 10 µg Gentamicin (GM), which suggest that based on this research, it can be used as a drug of choice.

Moreover, different susceptibility to antibiotics including 25 µg Chloramphenicol (C), 10 µg Streptomycin (S), 25 µg Tetracycline (T) and 25 µg ColistinSulphate (CO) and 200 µg Sulphatriad (ST) could potentially means presence of resistance genes in some of the isolates. This might be due to differences in sampling points and of course the good laboratory techniques employ ensuring minimal biological contamination.

CONCLUSION

In this research, different pseudomonads isolates were tested for resistance to antibiotics and the result shows diversity among their susceptibility behavior. This work potentially indicates that, although pseudomonads are members of the group *Pseudomonadaceae* their behavior to antibiotics may differ due to their ability to mutate easily under conditions that favors mutation. Similarly, study of this kind is important for researchers that are interested in manipulating the genetic makeup of *Pseudomonas* spp and for tackling the well-known resistance ability of *pseudomonas* spp. Therefore, its recommended that strains with higher zone of inhibition be further explode and study their minimum inhibitory concentrations for further evaluation.

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