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Antibiogram Characteristics and Associated Resistance Genes of Commensal *Pseudomonas* species Isolated from Soil and Plant Rhizosphere in the Eastern Cape Province, South Africa

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The prevalence, antibiogram characteristics and associated antibiotic resistance genes of commensal *Pseudomonas* species isolated from soil and plant rhizosphere in the Eastern Cape province of South Africa were investigated. Polymerase chain reaction (PCR) based procedure was used to identify the isolates and screened for antibiotic resistant genes. Seasonal distributions of isolates were as follows: summer (70.59%), winter (20.59%), autumn (6.86 %) and spring (1.96%). During summer, most of the *Pseudomonas* isolates were recovered from plant rhizosphere (95.8%), followed by plant root (2.8%) and cultivated soil (1.4%). *Pseudomonas putida* was the most prevalent *Pseudomonas* species and distributed in the following order: plant rhizosphere (95%); plant root (41.2%); and cultivated soil (20%). Antibiogram of the isolates revealed 100% resistance to clinamycins, trimethoprim, vancomycin and oxacillin. About 92.9% of the isolates from plant roots; 85% from plant rhizosphere; and 75% from cultivated soils were susceptible to gentamicin. Ofloxacin showed activity against the isolates from plant root (78.6%), cultivated soil (75%) and plant rhizosphere (55%). Ciprofloxacin was also active against isolates from cultivated soil (75%), plant root (64.3%) and plant rhizosphere (63.8%). Integron gene was detected in all the *Ps. aeruginosa* isolates, and in about 30% of *Ps. putida,* and 6.3% of the unspeciated *Pseudomonas* species. The presence of integron gene in some of the *Pseudomonas* strains is of environmental and public health concern as it suggests the *Pseudomonas* isolates to be potential reservoirs of antibiotic resistance determinants in the environment.

Key words: *Pseudomonas* species, Resistance determinants, Plant rhizosphere, Integron gene, Public health.

Pseudomonas species are Gram-negative bacteria inhabiting soil, water, plants, and animals including humans, and they are renowned for their nutritional and ecological versatility. As opportunistic human pathogens, they are common causes of nosocomial infections especially as persistent infections in immunocompromised individuals[1], and some are capable of causing serious infections in non-mammalian host species such as insects[2], nematodes[3], and plants[4]. The effectiveness of bacteria in causing infection is likely due to a suite of well-regulated virulence factors and defense mechanisms such as multidrug resistance pumps[5], and biofilm formation[6]. It was originally hypothesized that the biofilm acted as a shield that protected the bacterial cells from harsh environmental conditions[6]. However, it is now believed that individual cells within the biofilm may

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have an altered metabolism that renders them more resistant to environmental stress and prolonged antibiotic treatment. Moreover, the biofilm does not prevent the entrance and diffusion of antibiotics[7, 8]. In contrast to these negative qualities of biofilm formation, *Pseudomonas fluorescens* has been reported to coat plant roots by forming a biofilm, which may protect roots against soil bacterial and fungal pathogens[9].

Rhizosphere is the zone of soil that is instantly adjacent to plant root that support high level of bacterial activity[10]. It is vital for plant health and nutrition[11, 12]. The rhizosphere harbours a large and varied community of prokaryotic and eukaryotic microbes which compete and interact among themselves and the plant root[11]. These interactions affect adaptation of plant and their susceptibility to disease[13]. It remains a challenge for plant and microbial biologists to understand the complexity of rhizospheric environment and how microbial community adapt and respond to changes in biological, chemical and physical properties of the environment[11, 13]. The soil is a vast reservoir of microbial population, and the rhizosphere has been reported to characteristically contain about two tons of microorganisms/acre. Microbial adaptation to soil conditions influences the genera and numbers of microorganisms found in soil environment[14].

Pseudomonas species are important components of the rhizosphere, and saprophytic *Pseudomonas* are common root-colonizing bacteria. Studies have shown that certain isolate can enhance plant health[11, 15], but the potential of rhizospheric *Pseudomonas* to cause disease and transmit resistant genes is yet to be ascertained. The use of antibiotics as growth promoter in agriculture and for the treatment of humans, animals and plant has become a universal practice[16]. Several antibiotics have been used including tetracycline, aminoglycosides, macrolides, fluoroquinolones, cephalosporin and sulfonamides[16, 17]. Understanding the sources of antibiotic-resistance genes is of great importance because human exposure to multi-drug resistant microbial contaminants can occur in a number of ways[18]. Land application of animal manure or biosolids produced from wastewater treatment plants may be among the major activities responsible for introduction of antibiotic-resistant bacteria and their genes in the environment. The ability of bacteria to develop multiple-drug resistance is due in part to their ability to acquire new antibiotic resistance genes. Mobile elements called integrons determine a site-specific recombination system that is responsible for the acquisition of many antibiotic resistance determinants[19, 20]. As part of our surveillance of commensal bacteria as reservoirs of antibiotic resistance determinants in the Eastern Cape Province, South Africa, we assess the prevalence, antibiogram characteristics and associated antibiotic resistance genes of commensal *Pseudomonas* species isolated from soil and plant rhizosphere in the Eastern Cape Province.

MATERIAL AND METHODS

Samples Collection

Samples were collected once a season to fairly represent the South African climatic seasons. Samples collected include soils from some vegetable and cultivated soil (Butter nut, spinach, cabbage, maize), as well as plant roots and rhizosphere (Spinach, Cabbage, Grass). Samples were immediately transported in cooler boxes to the laboratory for analyses.

Isolation and identification of Pseudomonas species

Fifty millilitres (50 ml) of sterile nutrient broth was inoculated with 5 g of cultivated soil samples. Plant root samples were carefully shaken to remove loosely adhering soil and rinsed with sterile distilled water[21]. About 5 g of the root samples were cut into smaller pieces with a pair of sterile scissors and inoculated into sterile nutrient broth[22], with modification. Also, 5 g of rhizosphere sample was weighed and inoculated into sterile nutrient broth[21, 23]. All inoculated broths were incubated overnight at 37°C and 150 rpm. At the end of incubation, about 5 µl of the cultures broth were streaked onto Glutamate Starch Phenol-red (GSP Agar) plates and incubated at 37°C for 24 h. Colonies that were Blue-violet in colour and surrounded by a red-violet zone on GSP agar were selected as presumptive *Pseudomonas* isolates and purified using the same isolation medium. Pure isolates were then transferred onto Nutrient agar plates and incubated overnight at 37°C. Thereafter, the pure isolates were subjected to Gram staining

and oxidase test. Only Gram-negative, oxidase-positive isolates were selected for biochemical identification using API 20 NE kit (bioMerieux, Marcy l'Etoile, France).

Isolation of genomic DNA

Isolation of genomic DNA from the bacterial isolates was done following the description of Sambrook and Russell[24]. Briefly, single colonies of the identified *Pseudomonas* species grown overnight at 37°C for 24 h on nutrient agar plates were picked, suspended in 200 µl of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using Dri-block DB.2A (Techne, SA) for 10 min at 100°C. The cell debris was removed by centrifugation at 11,000 × *g* for 5 min using a MiniSpin micro centrifuge (Merck, SA) and the supernatant used directly as template DNA for the PCR reaction or stored at -80°C until ready for use.

Polymerase chain reaction (PCR) confirmation of Pseudomonas genus

The genus specific primer pair PA-GS-F (52 -GACGGGTGAGTAATGCCTA-32) and PA-GS-R (52 -CACTGGTGTTCCTTCCTATA-32)[25] were used for the identification of *Pseudomonas* genus. PCR reactions were performed in 50 µl volume of reaction buffer containing 0.05 unit/µl Taq polymerase as recommended by the manufacturer (Fermentas Life Sciences) and 5 µl of DNA template. *Pseudomonas* reference strain ATCC 27853 was used as positive control while sterile Milli-Q PCR grade water (Merck, SA) was included in each PCR assay as a negative control. PCR conditions (Bio-Rad MyCycler™ Thermal Cycler) were as follows: 95°C for 5 min, 10 cycles of 94°C for 15 sec, 53°C for 30 sec and 72°C for 45 sec; this was repeated for another 25 cycles with the exception that the 72°C elongation step was increased by 5 sec every cycle. A final extension phase of 72°C for 10 min was used. The amplified products were held at 4°C after completion of the cycles. The amplified PCR products were electrophoresed in 0.8% agarose gels (Hispanagar, Spain) containing ethidium bromide 0.5 mg/l (Merck, SA) for 1 h at 100 V in 0.5 × TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and visualized and photographed with a imaging system Alliance 4.7 XD-79 (UVITEC Cambridge).

PCR Identification of Pseudomonas species

Species-specific primers sets for *Pseudomonas putida* (F1 5'-TCACCTCCGAGGAAACCAGCTTG-3'; F2 5'-TCTGTTGTGAACGCCCTGTC-3')[26], and *Pseudomonas aeruginosa* (F1 5¹-GGCGTGGGTGTGGAAGTC-3¹; F2- 5¹-TGGTGGCGATCTTGAAC TTCTT-3¹)[27] were used to speciate the *Pseudomonas* genus positive isolates identified earlier. All PCRs were performed in 50 µl volume of reaction buffer containing 0.05 unit/µl Taq polymerase as recommended by the manufacturer (Fermentas Life Sciences) and 5 µl of DNA template. The PCR condition (Bio-Rad MyCycler™ Thermal Cycler) for *Pseudomonas putida* was an initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 94°C for 30s, annealing at 55°C for 90s and extension at 72°C for 90s with a final extension at 72°C for 7 min. For *Pseudomonas aeruginosa* PCR condition was 95ºC for 1 min; 40 cycles of denaturation at 95ºC for 15 s, annealing at 58ºC for 20 s; final extension at 68ºC for 40 s. The amplified products were electrophoresed in 0.8% agarose gels (Hispanagar, Spain) containing ethidium bromide 0.5 mg/l (Merck, SA) for 1 h at 100 V in 0.5 × TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and visualized and photographed with an imaging system Alliance 4.7 XD-79 (UVITEC Cambridge).

Antibiotic susceptibility test

Antibiotic susceptibility testing was done in accordance with the descriptions of Bauer et al.[28] and CSLI[29] using antibiotics discs (Mast Diagnostics, Merseyside, UK) including Penicillins G (10 unit), Clinamycins (2 µg), Ciprofloxacin (5 µg), Rafamycin (5 µg), Trimethoprim (5 µg), Sulphamethoxazole (25 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Tetracycline (10 µg), Erythromycin (15 µg), Minocycline (30 µg), Vacomycin (30 µg), Cefotaxime (30 µg), Nalidixic acid (30 µg), Nitrofurantoin (30 µg), Cephalothin (30 µg), Ofloxacin (30 µg), Ampicillin (25 µg), Ampicillin-sulbactam (20 µg), and Oxacillin(10 µg) were employed. Interpretation of result was performed by measuring zones of inhibition and comparing with the interpretative chart to determine the sensitivity of the isolates to the antibiotics.

Antibiotic resistant genes

Polymerase chain reaction (PCR) was used to detect antibiotic resistant genes in the

Pseudomonas species using the specific primers sets as shown in Table 1. All PCRs were performed in total 50 µl volume of reaction buffer containing 0.05 unit/ µl Taq polymerase as-recommended by the manufacturer (Fermentas Life Sciences) and 5 µl of DNA template.-Cycling conditions (Bio-Rad MyCycler™ Thermal Cycler) were as follows: bla_{OXA} gene and $bla_{amp}C$ gene (94°C for 5min, 30 cycles of 25s of denaturation at 94°C, 40s of annealing at 53°C and 50s of extension at 72°C and a final cycle at 7 min at 72°C[30]; *Tet C* gene (3 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 65°C and 1 min at 72°C followed by 10 min at 72°C[31]; bla_{TEM} gene (3 min at 93°C, 40 cycles of 1 min at 93°C, 1 min at 55°C and 1 min at 72°C and finally 7 min at 72°C[32], integron gene (initial denaturation at 94°C for 12 min, 1 min of denaturation at 94°C, 1 min of annealing at 55°C and 5 min of extension at 72°C for a total of 35 cycles; five seconds were added to the extension time at each cycle[33]. The amplified PCR products were electrophoresed in 0.8% agarose gels (Hispanagar, Spain) containing ethidium bromide (EtBr) 0.5 mg/l (Merck, SA) for 1 h at 100 V in 0.5 × TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and visualized and photographed with a imaging system Alliance 4.7 XD-79 (UVITEC Cambridge).

RESULTS

Seasonal abundance and distribution of Pseudomonas isolates

A total of 120 presumptive *Pseudomonas* isolates were recovered on GSP agar of which 102 were confirmed as belonging to the *Pseudomonas* genus. These isolates were variously distributed among the sampling seasons. The highest number of isolates were recorded in summer (70.6%) followed by winter (20.6%), and the lowest in spring (1.6%). In autumn, 57.1% of *Pseudomonas* isolates were recovered from cultivated soil, 42.9% from plant root and none from plant rhizosphere (Table 1). In summer, 95.8% of the *Pseudomonas* isolates were recovered from plant rhizosphere, while 2.8% and 1.4% were recovered from plant root and

Table 1. Primers used in the detection of antibiotic resistant genes

Target genes	Primers (5'-3')	References
bla_{TEM} gene	AGGAAGAGTATGATTCAACA CTCGTCGTTTGGTATGGC	Wang *et al.*[57]
Tet C gene	GGT TGA AGG CTC TCA AGG GC GGT TGA AGG CTC TCA AGG GC	Agersø and Sandvang[58]
Integron gene	GGCATCCAAGCAGCAAG AAG CAG ACT TGA CCT GA	Fonseca *et al.*[54]
bla_{OXA} gene	TGAGCACCATAAGGCAACCA TTGGGCTAAATGGAAGCGTTT	Yang *et al.*[59]
$bla_{amp}C$	GGTATGGCTGTGGGTGTTA TCCGAAACGGTTAGTTGAG	Yang *et al.*[59]

Table 2. Seasonal distribution of *Pseudomonas* isolates

*Season	Sources		
	Cultivated soil	Plant root	Plant rhizosphere
Autuum (6.86 %)	57.14%	42.86 %	0 %
Winter (20.59 %)	0 %	57.14 %	42.85 %
Spring (1.96 %)	0 %	0 %	100 %
Summer (70.59 %)	1.38 %	2.77 %	95.83 %

*Summer (November to March); autumn (April to May); winter (June to August); spring (September to October).

Table 3. Antibiogram profile of *Pseudomonas* isolates

Antibiotics	Sources								
	Plant root n=14			Cultivated soil n=4			Plant rhizoshere n=80		
	S	I	R	S	I	R	S	I	R
Penicillins	0	0	14(100)	0	0	4(100)	0	1(1.25)	79(98.75)
Clinamycins	0	0	14(100)	0	0	4(100)	0	0	80(100)
Ciprofloxacin	9(64.29)	1(7.14)	4(28.57)	3(75)	1(25)	0	51(63.75)	14(17.5)	15(18.75)
Rafamycin	0	1(7.14)	13(92.86)	0	0	4(100)	1(1.25)	0	79(98.75)
Trimethoprim	0	0	14(100)	0	0	4(100)	0	0	80(100)
Sulphamethoxazole	0	0	14(100)	0	0	4(100)	3(3.75)	1(1.25)	76(95)
Gentamicin	13(92.86)	0	1(7.14)	3(75)	1(25)	0	68(85)	5(6.25)	7(8.75)
Chloramphenicol	0	0	14(100)	2(50)	1(25)	1(25)	1(1.25)	6(7.50)	73(91.25)
Tetracycline	0	1(7.14)	13(92.86)	0	2(50)	2(50)	1(1.25)	24(30)	55(68.75)
Erythromycin	0	0	14(100)	0	0	4(100)	2(2.50)	2(2.50)	76(95)
Minocycline	2(14.28)	6(42.86)	6(42.86)	1(25)	2(50)	1(25)	15(18.75)	36(45)	29(36.25)
Vacomycin	0	0	14(100)	0	0	4(100)	0	0	80(100)
Cefotaxime	2(14.28)	5(35.71)	7(50)	0	1(25)	3(75)	11(13.75)	43(53.75)	26(32.19)
Nalidixic acid	1(7.14)	0	13(92.86)	0	3(75)	1(25)	1(1.25)	2(2.50)	77(96.25)
Nitrofurantoin	0	0	14(100)	0	0	4(100)	3(3.75)	4(5)	73(91.25)
Cephalothin	0	1(7.14)	13(92.86)	0	0	4(100)	0	1(1.25)	79(98.73)
Ofloxacin	11(78.57)	2(14.28)	1(7.14)	3(75)	1(25)	0	44(55)	15(18.75)	21(26.25)
Ampicillin	0	0	14(100)	0	0	4(100)	0	3(3.75)	77(96.25)
Ampicillin-sulbactam	1(7.14)	2(14.28)	11(78.57)	0	1(25)	3(75)	5(6.25)	3(3.75)	72(90)
Oxacillin	0	0	14(100)	0	0	4(100)	0	0	80(100)

Values in parenthesis represent (%) occurrence of resistance (R); intermediate (I) and susceptibility (S) of *Pseudomonas* isolates

Table 4. Distribution of *Pseudomonas* species among grouped sources

Pseudomonas Isolate	Sources		
	Plant soil	Cultivated root	Plant rhizosphere
Ps. putida	41.2%	20%	95%
Ps. aeruginosa	0%	0%	2.5%
Other *Pseudomonas* spp.	58.8%	80%	2.5%

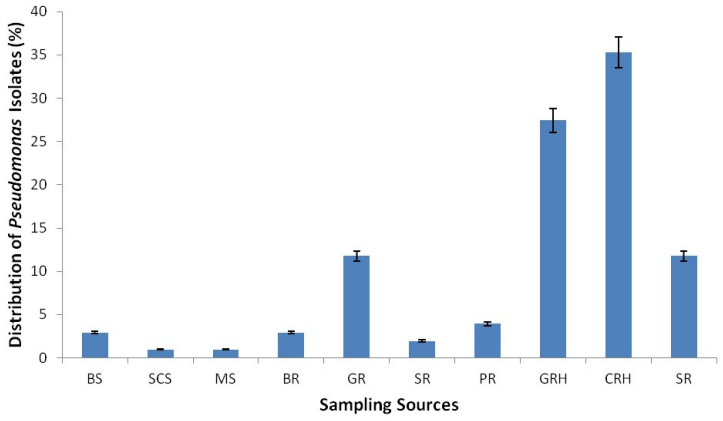
cultivated soil respectively. However, during spring, *Pseudomonas* isolates were only recovered from plant rhizosphere with none from either cultivated soil or plant root (Table 1). In general, the highest recovery rate (35.3%) of *Pseudomonas* isolates were obtained from cabbage rhizosphere, followed by grass rhizosphere (27.5%), and spinach rhizosphere and grass root (27.5%), while the least recovery rates of (0.98%) were from spinach soil and maize soil as shown in Fig. 1.

Antibiotic susceptibilities

Table 2 shows a summary of antibiotic susceptibility patterns of the *Pseudomonas* isolates. Isolates from plant roots were all resistant to penicillin, clindamycin, oxacillin, trimethoprim, chloramphenicol, erythromycin, nitrofurantoin, ampicillin and vacomycin. Variable susceptibilities were observed for the following antibiotics; gentamicin (92.9%), ciprofloxacin (64.3%), ofloxacin (13.8%), nalidixic acid (7.1%), minocycline and cefotaxime (14.3%). Also, 75% of the isolates from cultivated soil were susceptible to ciprofloxacin, gentamicin and ofloxacin, while 50% and 25% were susceptible to chloramphenicol and minocycline respectively. All the isolates from cultivated soil were resistant to rifampicin, penicillin, clinamycin, trimethoprim, erythromycin, oxacillin and ampicillin, while between 25 and 75% of the isolates were resistant to ampicillin-sulbactam, tetracycline and nalidixic acid. With respect to the isolates recovered from plant rhizosphere 98.8% were resistant to penicillin G while 1.3% showed intermediate response. Ciprofloxacin was susceptible to 63.8% of the isolates, while gentamicin and ofloxacin were susceptible to 85% and 55% of the isolates respectively.

Species-specific identification and antibiotic resistance gene cluster

Speciation of the *Pseudomonas* isolates by PCR revealed the following distribution: *Pseudomonas putida* (82.4%); *Ps. aeruginosa* (1.96%); and the remaining 15.7% grouped as other



Legend: BS (Butternut Soil); SCS (Spinach Cultivated Soil); MS (Maize Soil); BR (Butternut Root); GR (Grass Root); SR (Spinach Root); PR (Plant Rhizosphere); GRH (Grass Rhizosphere); CRH (Cabbage Rhizosphere); SRH (Spinach Rhizosphere).

Fig. 1. Percentage distribution of *Pseudomonas* based on isolation sources

Pseudomonas species. *Ps. putida* distribution was as follows: 41.2% from plant root, 20% from cultivated soil and 95% from plant rhizosphere (Table 3). Among the antibiotic resistant genes determined, bla_{TEM}, bla_{OXA}, bla_{amp}C and *tet*C were not detected in any of the isolates, but integron gene was detected in 29.8% of *Ps. putida,* 100% of *Ps. aeruginosa* and 6.3% of the other *Pseudomonas* species

DISCUSSION

Pseudomonas species are noted for their metabolic versatility and exceptional ability to adapt to and colonize a wide variety of ecologic environments, but also for their intrinsic resistance to a wide variety of antimicrobial agents. In this study, *Pseudomonas* isolates were recovered from cultivated soil, plant root and rhizosphere samples. Samples were collected by seasons to shed light on effect of season on *Pseudomonas* distribution in the community. The detection of *Pseudomonas* species all through the seasons supports their versatility and ability to proliferate under a wide range of climatic conditions. The different sample sources showed varied bacterial distribution throughout the sampling seasons. Garbeva et al.[34] revealed how different agricultural regimes strongly influence the structure of *Pseudomonas* population dynamic in soil. Our study suggests that seasons and different sample sources may play a role in the distribution of commensal *Pseudomonas* species in the environment. Similar findings have also been reported where plant species, seasonal variation and sampling site was shown to significantly affect the population dynamic of *Pseudomonas* in rhizosphere soils[35]. Berg et al.[36] reported a higher number of opportunistic pathogen in the rhizosphere when compared to bulk soil which supports our finding. A higher occurrence of *Pseudomonas* isolates was observed in rhizosphere samples compared to plant root and cultivated soil samples. The rhizosphere is rich in organic matter which stimulates microbial growth and favours the growth of human pathogens[11] which is of immense significance to human health.

The environment is significantly affected by the presence of antibiotic-resistant bacteria and associated genes. An emerging threat to public and environmental health has been reported and linked to the growing evidence of increasing antibiotic resistance in benign and pathogenic bacteria[37, 38]. The antibiotic resistance profile of *Pseudomonas* isolates observed in this study suggests that the studied microenvironment (cultivated soil, plant root and rhizosphere) is a potential reservoir of antibiotic resistance determinants in the environment. Also, most *Pseudomonas* isolates were observed to be resistant to major group of antibiotics. Berg et al.[39] demonstrated that some environmental isolates exhibited a high level of resistance to imipenem â-lactam group. Many of the *Pseudomonas* strains investigated in our present study were isolated from the plant rhizosphere samples. This particular microenvironment is rich in nutrients due to the exudation of organic compounds from plants[40]. Thus, the competition between microorganisms for these ecological sites is very high.

Previous studies[41, 42, 43] have revealed that bacteria isolated from environment with elevated levels of pollutants exhibit greater resistance towards antibiotics. The ecological value of the sensitivity or resistance of our environmental isolates to antibiotics lies in their ubiquitous presence in many habitats, in their capacities to display some level of multi-drug resistance (MDR). This study revealed some degree of multiple antibiotic resistances amongst the isolates recovered from cultivated soil, plant root and rhizosphere microenvironments (Table 2). It has been suggested that manure may introduce antibiotic resistance genes into the soil, which could enhance horizontal gene transfer to soil bacteria because it provides nutrients for activation of transfer and helps in mobilizing genetic elements[44, 45]. The presence of the antibiotic-producing bacteria and their resistance genes in nutrient-enriched environment could be one of the reasons for the occurrence of antibiotic resistance genes in the soil environment. The antibiotics produced can help the soil bacterial community to acquire resistance genes under a natural selection process[46]. The genetic diversity and natural characteristics of soil microenvironment play important roles in minimizing the effect of the introduction of genes in soil environment by manure application to enhance agricultural products. Based on our observed occurrence of

antibiotic resistance genes in the soil environment it is clear that there are regional differences in the diversity of antibiotic resistance gene pools that might be responsible for these variations. Our findings are supported by previous studies[47, 48, 49], which demonstrated that effluents can influence the ecological functioning of aquatic as well as soil milieu and increase the proliferation of more tolerant species showing a multiple antibiotic resistance profile. The study revealed that rhizosphere isolates had more discernible pattern of multiple-drug resistance.

This study also revealed the presence of integron gene in some of the *Pseudomonas* isolates and absence of other antibiotic resistance gene screened in all the isolates. The integron gene cassettes are proficient in transferring antibiotic resistance genes in the environment. This could imply that the efflux mechanism for resistance to integron determinant in *Pseudomonas* populations is dominant over the ribosomal protection mechanism. An interesting property of ribosomal protection is that it does normally confer high-level beta-lactam and tetracycline resistance. Consequently, the ribosomal protection mechanism of resistance might not provide much survival value in the presence of tetracycline in nature for the enteric bacteria[50]. It is well-known that multiple mechanisms are related to antibiotic resistance in *Pseudomonas* species[51], and the integrons role in this species is an additional element in the dynamics of the resistance acquisition. The presence of integron gene in *Pseudomonas putida* in this study is worrisome although, *Pseudomonas putida* is not commonly implicated in human infection but, recent reports has shown its involvement in hard to treat infection due to multidrug resistance[52, 53]. These integron could act as means for the dissemination of antibiotic resistance determinant among environmental and commensal bacteria.

Although previous studies[54] have addressed the prevalence of specific antibiotic resistances in the clinical milieu, the observation that intergon gene was present in the apparent absence of antibiotic selective pressure raises the question of how resistance persists. A study suggested that bacteria may have been able to adapt to the load of carrying resistance with little or no cost to their fitness[55]. In this state, the antibiotic-resistant microbiota would successfully compete with the sensitive phenotypes even in the absence of selection, which would make control of antibiotic resistance even more difficult. Indeed, many of the members of the bla_{OXA} â-lactamase family have been found in bacterial isolates originated from some European countries[56]. It is not certain whether these countries represent foci of strains harboring these enzymes, but in our data we did not find the presence of this bla_{OXA} gene in our *Pseudomonas* isolates. It may be suggested that these genes are restricted to clinical environment. The occurrence of highly drug-resistance *Pseudomonas* isolates in the environment has serious implications in a country with an ever increasing immune-compromised population. The presence of integron gene observed in *Pseudomonas* isolates, suggests that contact with still unknown bacterial reservoir may contribute to the evolution of *Pseudomonas* towards multidrug-resistance in South Africa. The presence of mobile genetic elements capable of transferring antibiotic resistance genes in the environment can become a major cause of broad-spectrum â-lactam resistance among environmental pathogens. Surveillance programmes to monitor the prevalence and spread of antibiotics resistance determinants in South Africa is thus essential. Information obtained would be useful in determining the efficacy of current infections control measures and antibiotics regimens.

CONCLUSION

This study confirms the ubiquity of *Pseudomonas* species and in particular, the predominance of the commensal *Pseudomonas putida* in all the environmental samples assessed including plant rhizosphere. The dominance of *Pseudomonas* isolates in plant rhizosphere also potentiates the rhizosphere as an environment which favors microbial growth. The multiple antibiotic resistance observed in the *Pseudomonas* isolates, demonstrates the intrinsic resistance of *Pseudomonas* to several antibiotics. The presence of integron genes in the isolates reveals that the studied microenvironment is a potential reservoir of antibiotic resistant genes. The absence of some of the antibiotic resistance gene screened despite the phenotypic resistance observed suggest the need for further investigation. This work represents

the first report on the antibiogram characteristics of commensal *Pseudomonas* species isolated from some environmental sources in the Eastern Cape Province, South Africa.

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