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[1]The Oke- Ogun Polytechnic, Saki Oyo State Nigeria, Department of Science Laboratory Technology, Microbiology option.
[2]Ladoke Akintola University of Technology, Ogbomosho, P.M.B 4000, Department of Pure & Applied Biology.
[3]Department of Microbiology and Botany, University of Ibadan, Ibadan, Nigeria.
[4]Department of Zoology, Parasitology Unit, University of Ibadan, Nigeria.
[5]Cellular Parasitology Unit, Department of Zoology, University of Ibadan, Nigeria.
[6]Department Food Science Technology, the Oke-Ogun Polytechnic, Saki.
[7]Lead City University, Ibadan, Faculty of Medical Science, Chemical Science (Biochemistry Unit).

Contacts of Authors

*** To whom correspondence should be addressed: Adeoti Olatunde Micheal**

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Predictive comparative antibiotic resistance (AMR) profiles of rhizobacteria genes using CARD: a bioinformatics approach

Adeoti Olatunde Micheal[1,2,5]*, Oni Abosede Catherine[1], Adeoye Kafilat Adenike[1,4], Adeoti Oluwole Adeola[6], Adeoye Basirat Adedamola[7] and Adesina David Ademola[1,3]

Abstract

Members of the Plant Growth Promoting Rhizobacteria (PGPR) have been severally implicated as excellent growth enhancers, yield promoters as well as bio-fertilizers. A study on antibiotics surveillance of PGPR is urgently needed as caution towards its continued usage in Bio-science and Agro-allied. Antimicrobial resistance has become a great concern in agriculture and public health. The detection and characterization of antimicrobial resistance move from targeted culture and enzyme-based reaction to high-throughput metagenomics; acceptable resources for the analysis of large-scale information area unit as an expected rescue. The excellent bioinformatics tool newly curated for Antibiotic Resistance information (CARD; https://card.mcmaster.ca) could be a curated hub and resource-providing-referenced server for deoxyribonucleic acid and protein sequences as well as detection models on the molecular radar for antimicrobial resistance. This study employed CARD as pathogenomics repertoires for high-quality reference information on retrieving antibiotics resistance information on twenty-two carefully-selected members of Rhizobacter from NCBI. NCBI and CARD on-line platform were employed in polishing of antiobitics resistance info of selected PGPR genera such as *Leguminosarum*, Azotobacter, Azospirillum, Erwinia, Mesorhizobium, Flavobacterium *Paenibacillus Polymyxa*, *Bacilli mycoides*, *B. subtilis*, and *Burkholderia pseudomallei* among others. The data generated showed evidence that these rhizobacteria could be resistant to certain drug classes under a different Antimicrobial Resistance (AMR) Gene families using different phyto-pathogenic genes (ARO terms) using different resistance mechanisms. This distinctive platform provides bioinformatics tool that bridges antibiotic resistance considerations, which could be a fallback for policies in healthcare, agriculture and the environment.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR), Comprehensive Antibiotic Resistance Database (CARD), Antimicrobial Resistance (AMR), Phyto-pathogenic, Metagenomics, Bio-fertilizers

Introduction

Rhizobacteria are a group of agronomic bacteria that form a mutualistic symbiotic association, which is beneficial to both parties. Members of rhizobacteria are an essential group of microorganisms used as bio-fertilizer [1, 2, 3] and plant growth promoters, which are often referred to as PGPRs [4, 5, 6]. PGPR enhance plant growth by adding nutrients which act as inoculants during bio remediation, phytostimulation and biological control are classified based on their essential roles in the plant they inhabit [7, 8, 9]. The most common of PGPR species which are present in the rhizosphere are members of the genus *Azospirillum* [10, 11], Bacillus spp. and *Pseudomonas spp* [12, 13, 14, 15]. The increasing reliance on plants as major sources of pharmaceuticals, cosmetics, and fragrance flavours to meet with the increasing rise in the world population hence the urgent need to investigate its potential antibiotic potentials if barriers of its non-pathogenicity are trespassed [16,17,18].

Recent advancements in pathogenomics have advanced the phenomenon of antimicrobial resistance has gained benchmarking astute as a world number one public health threat [19, 20]. Therefore, efforts should be dissipated toward the characterization of antimicrobial resistance with increasing attention at the international level and proven to global acceptance in recent United Nations radars. The enzyme based polymerase chain reaction (PCR) characterizes and effectively serve as indicator of pathogenic microorganisms such as *Escherichia coli, Salmonella.* Non-pathogenicity is associated with Antimicrobial Resistance whereas culture- and PCR-based may have provided the necessary sigh into the prevalence of resistance. These techniques had thus enhance our ability to review both the evolution and ecology of antimicrobial resistance among the entire microbiota population level [21, 22].

There are several web servers exist nowadays that completely identify ontological AMR genes. These customized *in silico* resources are primarily designed for screening of one ordination or many assembled contigs of Antibiotic Resistance metaphysics (ARO) developed by excellent antibiotic resistance information [21], which is a notable improvement in AMR bio curation. Such an annotational classification is incredibly useful for useful description. The CARD includes bioinformatics tools that modify the identification of antibiotic resistance genes from whole- or partial-genome sequence information together with unannotated raw sequence assembly contigs [23].

Over the ages, because of the evolutionary and ecological drift, there are many bacteria of different genera that have migrated beyond the coast of their ecological niches in an attempt to seek new abode and survival. Some of these organisms include members of *Pseudomonas, Serratia,* and *Escherichia,* which have variously acquired resistance complexity along the wheel of change. With this in mind, PGPRs are beneficial today because of their various roles in agriculture and food security, hence the need for predictive genomic resistance profile as a tool for antibiotic resistance for future age.

The present study is an *in silico* surveillance attempt to predict AMR molecules among farmers friendly PGPR bacteria, although presently environmental opportunistic isolates. PGPR influence plant growth in two different ways: direct and indirect. The direct promotion of plant growth by PGBR is by the synthesis of phyto-hormones, thereby facilitating the uptake of certain nutrients from the environment. Indirect plant growth promotion occurs when PGBR lessen or the risk of phytopathogenic organisms, making them serve as antibiotic by producing bio-control synthesis. We aimed at using bioinformatics tool to predict the antibiotic resistance genes in PGBR.

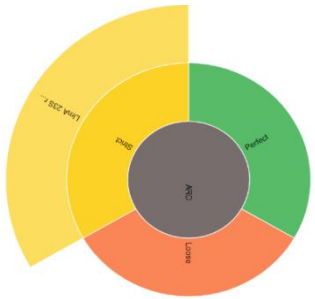
Materials and Methods

Retrieval of complete genomes from NCBI: To analyze antimicrobial resistance genes, twenty-two complete genomes of selected Plant Growth Promoting Rhizobacteria (PGPR) complete sequences were randomly retrieved from NCBI database. The retrieved sequences were in FASTA format, which was copied from the National Center for Biotechnology Information NCBI) website; https://www.ncbi.nlm.nih.gov as well as their accession numbers of PGPR.

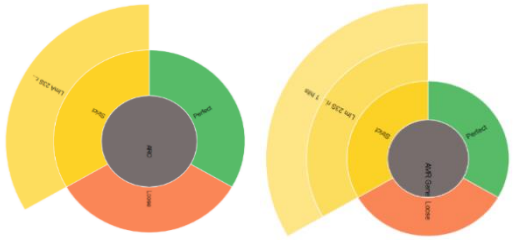
Analyzing nucleotide sequence on CARD: Nucleotide sequences of twenty two members of PGPR was imported into the CARD analyzing software from genbank using custom software developed specifically for the retention of all annotations, NCBI accession numbers and taxonomy identification (ID) numbers of the PGPR. The importation of these follows a process in which sequences were first acquired from genbank in FASTA format (https://www.ncbi.nlm.nih.gov/) and then loaded into the CARD's Chado database [24]. By convention, CARD uses only the subset of the available NCBI that is relevant to antibiotic-resistant bacteria. Individual Antibiotic Resistance Ontology (ARO) terms in the CARD have been associated with specific computational tools and models [25, 26, 14].

Results

Chart representation of RGI results for AMR genes and AMR family as retrieved by CARD on 22 selected PGPR (Figure 1).

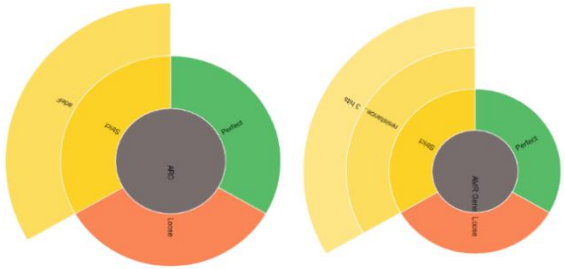


a: AMR gene family of CP0422721.1



b: AMR gene family of CP021965.1

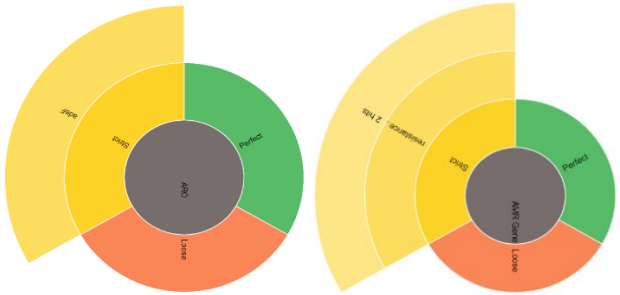
c: AMR Gene family of CP045291.1



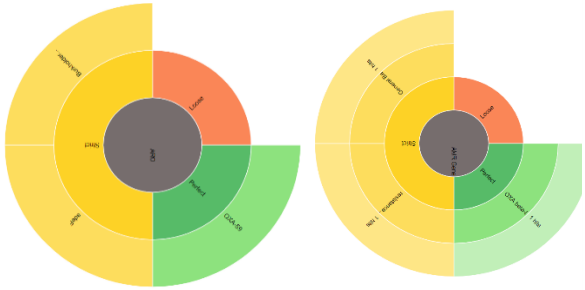
d: AMR gene family of CP019171.1

e: AMR gene family for CP010313.1

f: AMR gene family of CP029603.1



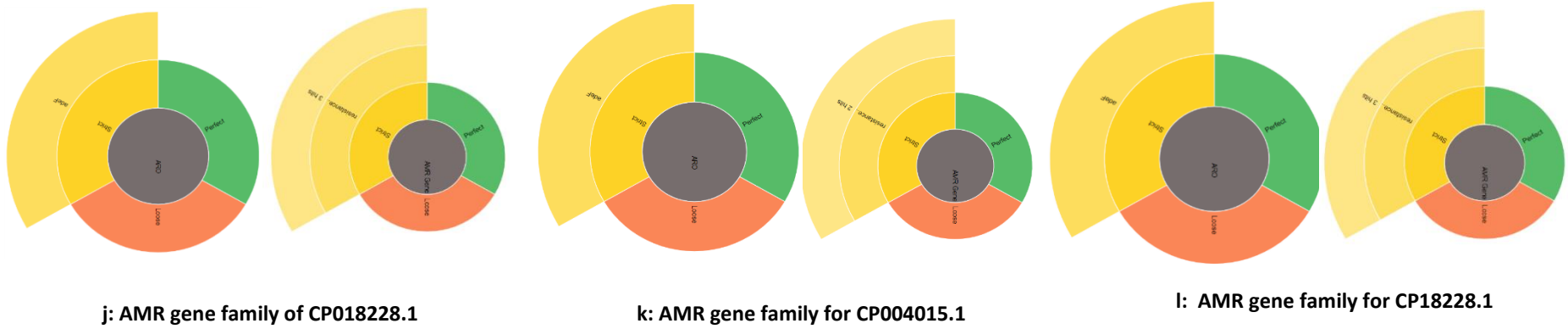
g: AMR gene family for CP033430.1



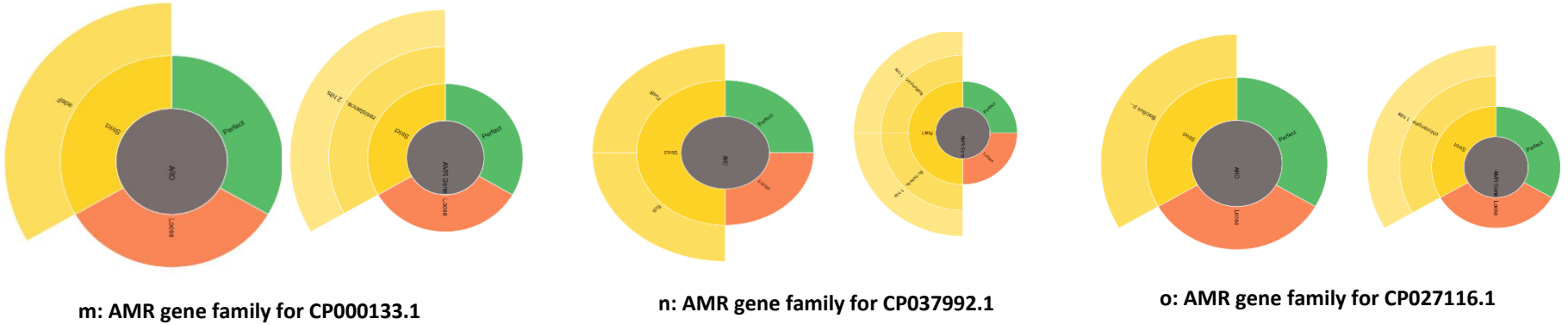
h: AMR gene family of BX571966.1

i: AMR gene family of CP040552.1

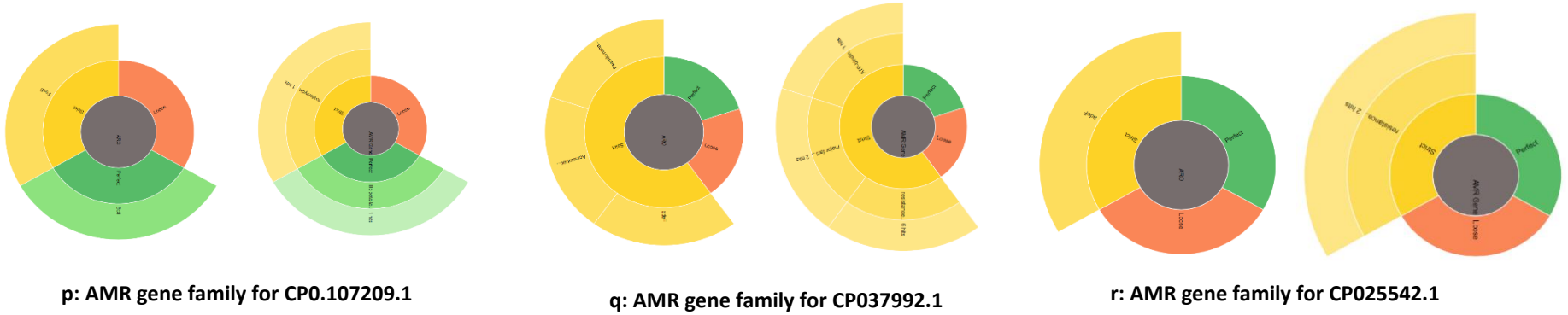
Figure 1. Continued



j: AMR gene family of CP018228.1 k: AMR gene family for CP004015.1 l: AMR gene family for CP18228.1



m: AMR gene family for CP000133.1 n: AMR gene family for CP037992.1 o: AMR gene family for CP027116.1



p: AMR gene family for CP0.107209.1 q: AMR gene family for CP037992.1 r: AMR gene family for CP025542.1

Figure 1. Continued

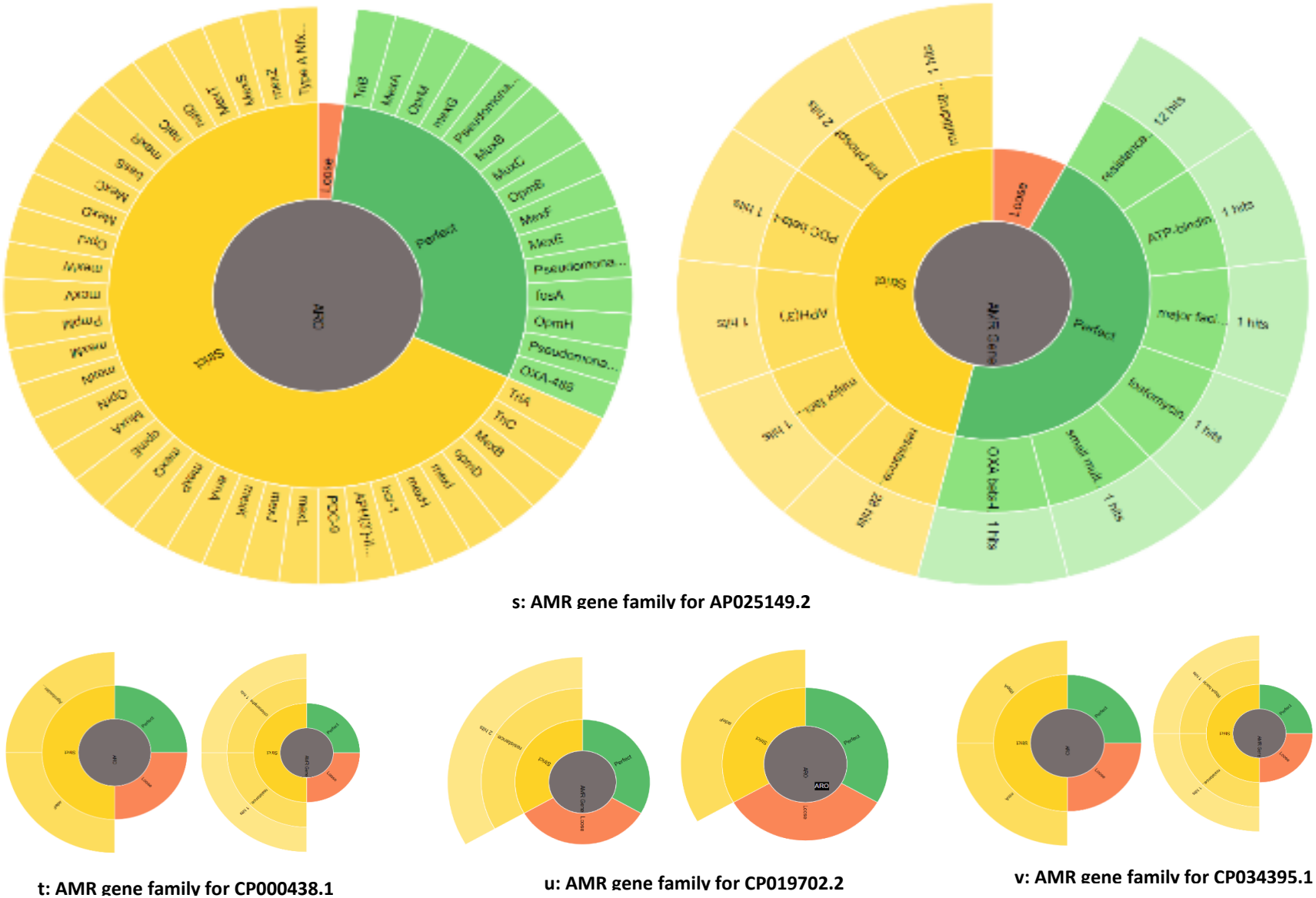


Figure 1. ARO classification tags for the selected twenty-two PGRD bacteria a-v: The assemly contigs retains the RGI into perfect (green), strict (flexible)and loose(spurious partial) resistomes.

Table 1. Prevalence of AMR gene family among PGPRs.

	Accession No	Prevalence of AMR genes		
		Perfect (%)	Strict (%)	Loose (%)
1	CP 042272.1	33	34	33
2	CP021965.1	30	40	30
3	CP045291.1	20	60	20
4	CP019171.1	35	35	30
5	CP010313.1	25	50	25
6	CP029603.1	25	50	25
7	CP033430.1	30	40	30
8	BX571966.1	25	50	25
9	CP040552.1	25	50	25
10	CP018228.1	30	40	30
11	CP004015.1	44	28	28
12	CP018228.1	23	38	29
13	CP000133.1	35	30	35
14	CP037992.1	25	50	25
15	CP027116.1	33	43	24
16	AP007209.1	40	25	35
17	CP037992.1	23	54	23
18	CP025542.1	13	74	14
19	CP025149.2	38	38	24
20	CP000438.1	48	48	04
21	CP019702.2	25	50	25
22	CP034395.1	34	44	22

Discussion

This study employed CARD ARO and AMR resistomes classification in agreement with earlier studies. The RGI genes in CARD predict resistomes for genomic and metagenomics data for gene mutations from NCBI retrieved nucleotide sequences by using a combination of open reading frame prediction [29, 30]. In consonance with earlier studies, in this study, the perfect algorithm predicted AMR proteins with exact homology (100%) with a query on the CARD reference sequences. In the same vein, members of PGPR on CARD algorithm showed strict genes under AMR/ARO curation because strict RGI genes are more flexible by allowing flexible variation from the genome reference sequence within the curated BLAST cut-off, which is useful for detection of previously unreported variants of antibiotics target changes through altered sequences [31].

The loose algorithm under CARD was an indication of resistance genes, which work outside the detection of target, which could enable the detection of new, emerging risks and more distant homologs of antibiotics resistance genes. The loose portends a computational novel AMR gene discovery [32]. The study is somewhat pioneer that employed CARD as surveillance algorithm for PGPR, it is however a novel study because all the twenty-two selected PGBR clearly showed the three AMR variants into perfect, strict and loose. This is an outright indication that the bacteria under study harbour resistance genes. The degree of prevalence of these genes varies among the PGBR. In consonance with earlier studies, drug efflux accounted for 67% as the mechanism of gene resistance with other mechanism. This study is in contrast with earlier studies leading to curation paradigm of CARD resistomes, which operates on four primary AMR gene family to, which resistance is conferred on specificity and sensitivity of antibiotics, which rest on the experimental interpretation of Minimum inhibitory concentration of the antibiotics. This study implicated LimA 23SrRNA methyltransferase in *Paenibacillus Polymyxa* and *P. odorifer* strain responsible for resistance to lincosamide antibiotics, while adeF genes are predictably responsible for resistance to fluoroquinolone and tetracycline antibiotics *Delftia spp* and *Bradyrhizobium* species.

The most variant genes (adeF, OXA-59, omp 38, amrA and amrB) of antibiotic resistance through antibiotic efflux pump. In the same vein, members of Rhizobium species use adeF genes for resistance to fluoroquinolone and tetracycline antibiotics family while Rhizobium species in this study use BcII, FosB genes for resistance to fosfomycin, cephalosporin, penam, phenicol antibiotics family [27, 28].

Conclusion

CARD as technological metagenomics tools here has further demonstrated its wider applicability as primarily better curation paradigm over ResFinder [31], ARG-ANNOT and even catalog of resistance alleles in NCBI [32]. The new CARD rules allow diversity inclusion of experimentally-proven data on axiomatic variation in agricultural or environmental isolates.

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