An *In-Silico* Analysis of Acquired Antimicrobial Resistance Genes in *Pseudomonas Aeruginosa* Plasmids

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Software article

Keywords: Pseudomonas aeruginosa, plasmids, AMR gene

Posted Date: September 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-870436/v1

Abstract

Introduction: The aim of this study to reveal the prevalence of acquired antimicrobial genes in sequences of *P. aeruginosa* plasmids by using *in silico* methods.

Methods: This study included 828 items with using *'Pseudomonas aeruginosa* and plasmid' keywords for searching in NCBI database. The sequences of 94 plasmids were retrieved from GenBank and analysed for detection of acquired antimicrobial resistance (AMR) genes with using ResFinder 2.1 database/webserver, KmerResistance 2.2 database. and ResFinderFG 1.0. Plasmids Sequence were aligned with using MEGA X Molecular Evolutionary Genetics Analysis across Computing Platforms.

Results: 67 out of 94 plasmids sequences were qualified as AMR containing plasmids. For detected 9 classes of AMR genes, aminoglycosides 39.6% were highest rate. The next frequencies were beta-lactams (19%), sulphonamides (14.5%), and fluoroquinolones (10.1%). For nine classes of antibiotics, 74 AMR gene were identified. Prevalent of sulphonamide resistance gene *Sul1 was* 32 out of 277 gene. In AMR plasmids, 6 ARD family were detected. 15 representative genomic sequences selected from each clade and three clades were revealed from them. The relationship of clades with drug resistance was not significant (p-value = 0.682).

Conclusions: Analyzing the Information in annotated sequence is reveal the mechanism of spreading of resistance gene in plasmids. Detection the trace of AMR genes in world papulation can help to find more response to question in regards to spread of infection and analysis of AMR gene sequences give more insight to scientist to control of *Pseudomonas aeruginosa* infections.

Introduction

Pseudomonas aeruginosa habitats are very wide including soil and aquatic environments. This species can cause diseases in humans, animals and plants. *P. aeruginosa* is highly prone to drug resistance mostly by means of plasmids. plasmids are tools for horizontal gene transfer (HGT), in bacterial genetics evolution and adaptation. These mobile genetic elements are very diverse and have a high range hosts and ecological niches. Based on genetic organization, size and host range, 14 incompatibility groups of plasmids have been detected in Pseudomonas[1, 2]. Incompatible groups are using same replication control. This groups are very mobile and capable to gene transfer called promiscuous. Promiscuity gave great role in antibiotic resistance spreading among bacterial populations [3].

Antimicrobial resistance (AMR) is a more growing issue in *P. aeruginosa* infections that cause health system crisis. The empirical use of antibiotics in veterinary and medicine promote and creating resistance plasmids that circulate in universe populations. Spread of plasmid harbor AMR genes as threat to public health and environment leads to mortality and morbidity. Antimicrobial resistance (AMR) in *P. aeruginosa* is a worldwide health problem and need more attention in diagnosis and finding the map of spreading by plasmids. We aimed in this study to reveal the prevalence of acquired antimicrobial genes in sequences of *P. aeruginosa* plasmids by using *in silico* methods.

Materials And Methods

Plasmid selection

This study included all *'Pseudomonas aeruginosa* plasmid. Overlay 828 item were found with using *'Pseudomonas aeruginosa* and plasmid' keywords for searching in NCBI database (Table 1). Based on web data this sequence has been published from 1991 until 2020. Size of plasmid ranged between 2140 bp _ 555265 bp and GC content were 30.1–65.8%.

Screening for acquired AMR genes in plasmids

The sequences of 94 plasmids were retrieved from GenBank. This sequence were conducted *in silico* analysis for detection of acquired antimicrobial resistance genes with using ResFinder 2.1 database/webserver which 67 plasmids has AMR genes (Table 1) [4]. Fifteen classes of antibiotic presented in database considered for screening that included, aminoglycoside (AG), beta-lactam, colistin, a fluoroquinolone (FQ), fosfomycin, fusidic acid, glycopeptide, macrolide-lincosamide-streptogramin B (MLS), nitroimidazole, oxazolidinone, phenicol, rifampicin (RP), sulphonamide (SM), tetracycline (TC), and trimethoprim (TP). Search setting parameters for all 15-drug classes were adjusted to 90% as a minimum for percent identity and 100% for perfect alignment, also 60% for minimum length of sequence nucleotides to overlap with resistant genes. DNA of plasmid were submitted into database online software.[5] Analysis results including, predicted phenotype of resistance gene, database accession number, starting contig position of the gene, and alignment high-scoring segment pair (HSP) query length, were gathered from program. All date were recorded in excel software. For all plasmids the index of potential multiple antibiotic resistance (p-MAR) were calculated based on screened 15 classes of antibiotics [6, 7]. The analysis of p-MAR data's, can classify plasmids into three groups as: multidrug-resistant (MDR), extensively drug-resistant (XDR) or pan drug-resistant (PDR) by using the previously reported standards [5, 8] This index is a good marker for epidemiology detection of isolates origin antibiotic use and the rate of 0.2 indicates a 'high-risk' of social health contamination [6, 9].

Verification of acquired AMR genes in Aeromonas plasmids

Further assessments of plasmid containing AMR genes were done with KmerResistance 2.2 database [10, 11]. Analysis was conducted with default setting including, 70% for identity threshold and 10% for depth correction. Results were saved on excel sheet and compared with the results of ResFinder program.

Probing of plasmids for antibiotic resistance determinants (ARD)

The resistance phenotype of gene were explored for plasmids with AMR genes with ResFinderFG 1.0 [12] server, that screened resistance with functional metagenomic antibiotic resistance determinants. This database server setting was considered 98% for per cent identity and 60% for minimum query length. Results which screened for more than 13, ARD families were saved in excel sheet as 'assembled contigs/genomes and sequences.

Phylogenetic analysis of the retrieved Plasmids genomes

Plasmids Sequence were aligned with using MEGA X Molecular Evolutionary Genetics Analysis across Computing Platforms (MEGA X; https://www.megasoftware.net/). software by ClustalW approach.

phylogenetic tree was done by a maximum parsimony (MP) approach in MEGA X software. Support value were considered 1,000 bootstrap replicates.

Statistical Analysis

Correlation between GC% and plasmid size were qualified with using XLSTAT with principal component analysis (PCA) analysis with setting included mean, standard deviation, and correlation (Pearson).

Connection of plasmid with containing AMR gene with sequences available in NCBI databases and similarity between them were determined using BLAST online tool. Sequences were aligned with MEGA X using likelihood model in considering bootstrap procedure (1000 replicates).

Results

Size and GC content of Pseudomonas *plasmids*

PCA were used to determine the relation between size and GC content, of 67 plasmids carrying AMR genes. Size of plasmid ranged between 2140 bp _ 555265 bp and GC content were 30.1–65.8%.

Table 1

Pseudomonas aeruginosa Plasmids contain AMR genes

strain number	Organism- plasmid	G + C %	size bp	AMR
PA1	Pseudomonas aeruginosa plasmid pP6qnrS1	51	117945	yes
PA2	Pseudomonas aeruginosa strain C plasmid pKLC102	60.9	103532	yes
PA3	Pseudomonas aeruginosa plasmid pKLC102	60.9	103532	yes
PA4	Pseudomonas aeruginosa strain NK546 plasmid pNK546b	57.1	232884	yes
PA5	Pseudomonas aeruginosa plasmid pUM505	60.9	123322	yes
PA6	Pseudomonas aeruginosa strain IP40a plasmid pIP40	51.3	167554	yes
PA7	Pseudomonas aeruginosa plasmid pR31014-IMP	56.4	374000	yes
PA8	Pseudomonas aeruginosa plasmid p12939-PER	57.3	496436	yes
PA9	Pseudomonas aeruginosa plasmid pJB12	62.6	30361	yes
PA10	Pseudomonas aeruginosa strain ST308 plasmid pCOL-1	60.2	31529	yes
PA11	Pseudomonas aeruginosa plasmid pA681-IMP	56.4	397519	yes
PA12	Pseudomonas aeruginosa plasmid p727-IMP	56.4	430173	yes
PA13	Pseudomonas aeruginosa plasmid pJB35	62.8	31166	yes
PA14	Pseudomonas aeruginosa plasmid Rms149	59.5	57121	yes
PA15	Pseudomonas aeruginosa strain FFUP_PS_37 plasmid pJB37	57.2	464804	yes
PA16	Pseudomonas aeruginosa strain P378 plasmid P378-IMP	50.5	51207	yes
PA17	Pseudomonas aeruginosa strain 10265 plasmid p10265-KPC	58.2	38939	yes
PA18	Pseudomonas aeruginosa strain PA1280 plasmid pICP-4GES	64.1	50914	yes
PA19	Pseudomonas aeruginosa strain 60512 plasmid p60512-IMP	62.7	24306	yes
PA20	Pseudomonas aeruginosa strain HS87 plasmid pHS87a	62.9	26825	yes
PA21	Pseudomonas aeruginosa strain COL-1 plasmid pNOR-2000	62.8	21880	yes
PA22	Pseudomonas aeruginosa strain AR441 plasmid unnamed2	60.9	57052	yes
PA23	Pseudomonas aeruginosa strain AR_0356 plasmid unnamed1	60.9	57053	yes
PA24	Pseudomonas aeruginosa strain PB353 plasmid pPB353_1	57.3	59923	yes
PA25	Pseudomonas aeruginosa strain PAcoop101 plasmid pCOOP-101	62.3	26108	yes
PA26	Pseudomonas aeruginosa strain S04 90 plasmid	57.7	159187	yes

strain number	Organism- plasmid	G + C %	size bp	AMR
PA27	Pseudomonas aeruginosa strain 15.2986 plasmid pPSTRAS1	56.5	9910	yes
PA28	Pseudomonas aeruginosa strain PA41437 plasmid pOXA-198	60.5	48978	yes
PA29	Pseudomonas aeruginosa strain PAB546 plasmid pNK546-KPC	57.2	475027	yes
PA30	Pseudomonas aeruginosa strain PA34 plasmid pMKPA34-1	57.2	95404	yes
PA31	Pseudomonas aeruginosa strain 14057 plasmid p14057-KPC	59.2	51663	yes
PA32	Pseudomonas aeruginosa strain HN39 plasmid pHN39-SIM	56.9	282042	yes
PA33	Pseudomonas aeruginosa strain HS87 plasmid pHS87b	60.7	11242	yes
PA34	Pseudomonas aeruginosa strain PABL048 plasmid pPABL048	56.6	141954	yes
PA35	Pseudomonas aeruginosa strain BH9 plasmid pBH6	63.5	41024	yes
PA36	Pseudomonas aeruginosa strain Y89 plasmid pY89	60.1	85842	yes
PA37	Pseudomonas aeruginosa strain K34-7 plasmid pK34-7-1	30.1	4440	yes
PA38	Pseudomonas aeruginosa strain AR441 plasmid unnamed3	57.1	438529	yes
PA39	Pseudomonas aeruginosa strain AR_0353 plasmid unnamed1	60.8	41559	yes
PA40	Pseudomonas aeruginosa strain AR_0356 plasmid unnamed2	57.1	438531	yes
PA41	Pseudomonas aeruginosa strain D5170990 plasmid pD5170990	60.3	32424	yes
PA42	Pseudomonas aeruginosa strain 163940 plasmid pTROUS1	56.4	42035	yes
PA43	Pseudomonas aeruginosa strain 121156 plasmid pNECK1	63.2	28859	yes
PA44	Pseudomonas aeruginosa plasmid pCB58	58.6	32207	yes
PA45	Pseudomonas aeruginosa strain PAG5 plasmid pPAG5	56.3	513322	yes
PA46	Pseudomonas aeruginosa strain PA121617 plasmid pBM413	56.4	423017	yes
PA47	Pseudomonas aeruginosa strain 1160 plasmid p1160-VIM	56.2	205426	yes
PA48	Pseudomonas aeruginosa strain ST463 plasmid p1011-KPC2	58.8	62793	yes
PA49	Pseudomonas aeruginosa strain PA-IMP-1 plasmid pYUI-1	58.2	21079	yes
PA50	Pseudomonas aeruginosa strain ST1006 plasmid pPA-2	55.5	7995	yes
PA51	Pseudomonas aeruginosa plasmid YLH6_p3	57.8	49162	yes

strain number	Organism- plasmid	G + C %	size bp	AMR
PA52	Pseudomonas aeruginosa strain FDAARGOS_570 plasmid unnamed	61.3	36032	yes
PA53	Pseudomonas aeruginosa strain TC4411 plasmid pPWIS1	57	419683	yes
PA54	Pseudomonas aeruginosa strain PA298 plasmid pBM908	56.9	395774	yes
PA55	Pseudomonas aeruginosa strain BH6 plasmid pBH6	55.9	3652	yes
PA56	Pseudomonas aeruginosa plasmid pMATVIM-7	65.8	24179	yes
PA57	Pseudomonas aeruginosa strain 2047 plasmid pPA2047	60.6	43660	yes
PA58	Pseudomonas aeruginosa strain C79 plasmid p1	58.1	40180	yes
PA59	Pseudomonas aeruginosa strain AR439 plasmid unnamed2	56.9	437392	yes
PA60	Pseudomonas aeruginosa strain CF39S plasmid pCF39S	56.6	468631	yes
PA61	Pseudomonas aeruginosa strain T2436 plasmid pBT2436	56.9	422811	yes
PA62	Pseudomonas aeruginosa strain T2101 plasmid pBT2101	57	439744	yes
PA63	Pseudomonas aeruginosa plasmid Birmingham IncP-alpha	61.8	60099	yes
PA64	Pseudomonas aeruginosa IncP-1alpha plasmid pBS228	59	89147	yes
PA65	Pseudomonas aeruginosa PA96 plasmid pOZ176	57.6	500839	yes
PA66	Pseudomonas aeruginosa PAO1 plasmid pAMBL2	60.4	24133	yes
PA67	Pseudomonas aeruginosa PAO1 plasmid pAMBL1	63.5	26440	yes

AMR genes for different drug classes

Detection of AMR gene has been done by screening the plasmid sequences in ResFinder database. This database provides testing the existing of the resistance gene by complete sequences that acquired horizontally. This database highly associated with phenotypic resistance detection[4, 5, 13]. For high occurrence of resistance gene identification, it was adjusted to default setting. 67 out of 94 plasmids sequences were qualified as AMR containing plasmids.

Table 2
frequency of AMR gene in Pseudomonas aeruginosa plasmids

AMR types		number
MDR	negative	29
	positive	38
	Total	**67**
XDR	negative	62
	positive	5
	Total	**67**
PDR	negative	65
	positive	2
	Total	**67**

For detected 9 classes of AMR genes as shown in Table 3, aminoglycosides 39.6% were highest rate. The next frequencies were beta-lactams (19%), sulphonamides (14.5%), and fluoroquinolones (10.1%). For nine classes of antibiotics, 74 AMR gene were identified. Prevalent of sulphonamide resistance gene *Sul1 was* 32 out of 277 gene as shown in Fig. 1.

Table 3

Resistance to different classes of antimicrobial drugs and p-MAR index found in a set of plasmids, the 40 plasmids listed showed the presence of different number of AMR genes to 9 drug classes. (Aminoglycoside = AG; Beta-lactam = BL; macrolide-lincosamide-streptogramin B = MLS; Phenicol = PH; Rifampicin = RP; Sulphonamide = SM; Tetracycline = TC; Trimethoprim = TP)

s/n	Organism/Plasmid	AG	BL	MLS	PH	FQ	RP	SM	TC	TP	Total	(p-MAR) index
1	Pseudomonas aeruginosa IncP-1alpha plasmid pBS228	1	1						1	1	4	0.266667
2	Pseudomonas aeruginosa PA96 plasmid pOZ176	8	4		2	6		2			22	0.333333
3	Pseudomonas aeruginosa PAO1 plasmid pAMBL2	2	3			1		1			7	0.266667
4	Pseudomonas aeruginosa plasmid Birmingham IncP-alpha	1	1						1		3	0.2
5	Pseudomonas aeruginosa plasmid p12939-PER	6	2					2			10	0.2
6	Pseudomonas aeruginosa plasmid p727-IMP	2	2	2	1	2	1	1	1	1	13	0.6
7	Pseudomonas aeruginosa plasmid pA681-IMP	3	3	2	1	2		4			15	0.4
8	Pseudomonas aeruginosa plasmid pBS228	1	1						1	1	4	0.266667
9	Pseudomonas aeruginosa plasmid pCB58	10	1			1		1			13	0.266667
10	Pseudomonas aeruginosa plasmid pJB12	5	1			1		1			8	0.266667
11	Pseudomonas aeruginosa plasmid pJB35	5	1			1		1			8	0.266667

s/n	Organism/Plasmid	AG	BL	MLS	PH	FQ	RP	SM	TC	TP	Total	(p-MAR) index
12	Pseudomonas aeruginosa plasmid pR31014-IMP	3	2	2	1	2	1	2	1	1	15	0.6
13	Pseudomonas aeruginosa plasmid R1033 transposon Tn1696	2			1			1			4	0.2
14	Pseudomonas aeruginosa strain 1160 plasmid p1160-VIM	5	1	3	1	3		2			15	0.4
15	Pseudomonas aeruginosa strain 121156 plasmid pNECK1	1	2			1		1			5	0.266667
16	Pseudomonas aeruginosa strain 163940 plasmid pTROUS1	1	2			1		1			5	0.266667
17	Pseudomonas aeruginosa strain AR_0356 plasmid unnamed2	4				2		2	2		10	0.266667
18	Pseudomonas aeruginosa strain AR439 plasmid unnamed2	12	2					2			16	0.2
19	Pseudomonas aeruginosa strain AR441 plasmid unnamed3	4				2		2	2		10	0.266667
20	Pseudomonas aeruginosa strain CF39S plasmid pCF39S	8				2		2	2		14	0.266667
21	Pseudomonas aeruginosa strain D5170990 plasmid pD5170990	9	1		1			1			12	0.266667
22	Pseudomonas aeruginosa strain FDAARGOS_570 plasmid unnamed	14	6		2	4		2			28	0.333333

s/n	Organism/Plasmid	AG	BL	MLS	PH	FQ	RP	SM	TC	TP	Total	(p-MAR) index
23	Pseudomonas aeruginosa strain FFUP_PS_37 plasmid pJB37	5	1			1		1			8	0.266667
24	Pseudomonas aeruginosa strain HN39 plasmid pHN39-SIM	1	2	1	1		1	1			7	0.4
25	Pseudomonas aeruginosa strain IP40a plasmid pIP40	1	1					1			3	0.2
26	Pseudomonas aeruginosa strain MRSN17623 plasmid pMRVIM0713	7	3		1	2		1			14	0.333333
27	Pseudomonas aeruginosa strain PA121617 plasmid pBM413	6	4	4	2	4		4			24	0.333333
28	Pseudomonas aeruginosa strain PA298 plasmid pBM908	4	6		2	4		2			18	0.333333
29	Pseudomonas aeruginosa strain PA34 plasmid pMKPA34-1	5	1		1			1	1	1	10	0.4
30	Pseudomonas aeruginosa strain PA41437 plasmid pOXA-198	4	1		1			1			7	0.266667
31	Pseudomonas aeruginosa strain PAB546 plasmid pNK546-KPC	1	1		1			1			4	0.266667
32	Pseudomonas aeruginosa strain PABL048 plasmid pPABL048	4	2					2			8	0.2
33	Pseudomonas aeruginosa strain PAcoop101 plasmid pCOOP-101	5	1					1		3	10	0.266667

s/n	Organism/Plasmid	AG	BL	MLS	PH	FQ	RP	SM	TC	TP	Total	(p-MAR) index
34	Pseudomonas aeruginosa strain PAG5 plasmid pPAG5	6	4	4	2	4	2	4		2	28	0.533333
35	Pseudomonas aeruginosa strain PB353 plasmid pPB353_1	4	6			4		2			16	0.266667
36	Pseudomonas aeruginosa strain PB354 plasmid pPB354_1	2	3			2		1			8	0.266667
37	Pseudomonas aeruginosa strain S04 90 plasmid	4	2					2			8	0.2
38	Pseudomonas aeruginosa strain T2101 plasmid pBT2101	8	12					8	4		32	0.266667
39	Pseudomonas aeruginosa strain T2436 plasmid pBT2436	10	4		2		2	4	2		24	0.4
40	Pseudomonas aeruginosa strain TC4411 plasmid pPWIS1	5	1					1			7	0.2

Antibiotic resistance determinants in Aeromonas plasmids

In AMR plasmids, 6 ARD family were detected. High frequency families were aminoglycosides acetyl-transferases and beta-lactamase with 38.30% and 31.91%, respectively as shown in Fig. 2.

phylogenetic analysis of plasmids

Complete genome sequences of 67 plasmid containing AMR genes were analyzed using software with consideration exclusion and inclusion criteria. Representative sequence from each group were selected with maximum parsimony tree approach. Finally, 15 representative genomic sequences selected from each clade were more analyzed (Fig. 3). As shown in Fig. 3 the evolutionary lineages and common ancestry of all plasmid sequences were revealed by the phylogenetic tree. Three clades were revealed from them. The relationship of clades with drug resistance was not significant (p-value = 0.682) as shown in Table 4.

Table 4
Distribution of MDR based on clades (p-value = 0.682)

	MDR		
Clade	**no**	**yes**	**Total**
1	13.33%	20.00%	33.33%
2	20.00%	6.67%	26.67%
3	20.00%	20.00%	40.00%
Total	**53.33%**	**46.67%**	**100.00%**

Statistical analysis of sequences

Online tools like as NCBI database, ResFinder 2.1 database/webserver, KmerResistance 2.2 database, ResFinderFG 1.0. and MEGA X Molecular Evolutionary Genetics Analysis were used for analyzing sequences and resistance gene. XLSTAT were used for PCA and statistical analysis.

Discussion

Antibiotic resistance is a result of selection pressure due to the overuse and frequent misuse of antibiotics in health systems. The profile of AMR gene in bacterial population is depend on horizontal transfer of mobile genetic elements. Plasmids are one of the major roles as mobile genetic elements. Specification of plasmid in bacterial give more insight in determination of ability of plasmid to transfer resistance gene and evaluation of genome. *Pseudomonas aeruginosa* plasmids has been sequenced and evaluated statistically, based on PCA analysis there was the negative Pearson's correlation ($r = -0.191$, $\alpha = 0.95$) between GC content and plasmid size. The negative correlation between plasmid specification may be due random or naturally transfer of genes among plasmids. Although there is correlation between genome size and guanine–cytosine (GC) content in bacteria, however, the this correlation not well explained [14].

The prevalence of AMR genes was screened in ResFinder database. Plasmids sequences were analyzed to detect acquired antimicrobial resistance genes. Disadvantage of this way is that the ResFinder database cannot find mutation, therefore it only detects acquired resistance genes. There is need to qualify resistance genotype with phenotypic identification. Investigation showed that there is very high correlation between phenotypic detection and AMR gene detection in ResFinder database, therefore, whole-genome sequences alignment is an alternative way to find drug resistance patterns [15]. In overall 71.28% of plasmid were containing AMR genes, of which, 38, 5, 2 isolates were MDR, XDR and PDR respectively.

The gene responsible for this resistance phenotype were aminoglycosides 39.6%, beta-lactams (19%), sulphonamides (14.5%), and fluoroquinolones (10.1%). For nine classes of antibiotics, 74 AMR gene were identified. Prevalent of sulphonamide resistance gene *Sul1 was* 32 out of 277 genes. Aminoglycosides are used for the treatment of *Pseudomonas aeruginosa* infections such as pulmonary infections in cystic fibrosis (CF) patients. This antibiotic is inactivated by enzymes that phosphorylate (aminoglycoside

phosphoryltransferase [APH]), acetylate (aminoglycoside acetyltransferase [AAC]), or adenylate (aminoglycoside nucleotidyltransferase [ANT] of which acetylation of aminoglycosides mainly occur at the 1, 3, 6', and 2' amino groups. The aminoglycosides resistance genes also transferred by means of plasmids[16]. The *Sul1* gene located in plasmids and products of this gene can degrade sulfonamides and trimethoprim antibiotics. Those are cheap and efficient antibiotics that have been used for a long time to treatment of human and animals' gram-negative infections [17]. Three genes *sul1, sul2* and *sul3* encode dihydropteroate synthase enzyme that inactivate sulfonamides and *sul1* gene is mostly transferred in associated with class 1 integrons and others by plasmids [18]. Quinolone resistance has been occur mostly by plasmids by tree mechanisms including (i) *qnr* genes that produce a quinolone-protective proteins, (ii) *aac*(*6'*)-*Ib* gene that produce a double class antibiotic-modifying enzyme which acetylates ciprofloxacin and norfloxacin and (iii) *qepA* gene that produce an efflux pump proteins [19].

Analyzing the Information in annotated sequence is reveal the mechanism of spreading of resistance gene in plasmids. Detection the trace of AMR genes in world papulation can help to find more response to question in regards to spread of infection and analysis of AMR gene sequences give more insight to scientist to control of *Pseudomonas aeruginosa* infections. MEGA X software were used for analyzing phylogenetic relationship between 67 plasmid containing AMR genes. Due to some restriction test were accomplished in three group and representative genomic sequences from each clade were more analyzed. Finally, three clades were revealed from analyzing representative sequences. There was no correlation of MDR with clades (p-value = 0.682)

Conclusion

This study results showed that there is no correlation between size and GC content of *Pseudomonas aeruginosa* plasmids. Most of plasmid carrying AMR genes that acquired horizontally. Three phylogenetically clade was revealed by molecular epidemiology software but there were not associated with drug resistance.

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors have no conflict of interest.

Funding

Not applicable

Authors' contributions

RR did all process including doing project and manuscript writing, editing and submitting.

Acknowledgements

The authors thank research deputy of Ardabil university for supporting this project.

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Conflict of interest

The authors have no conflict of interest.

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Figures

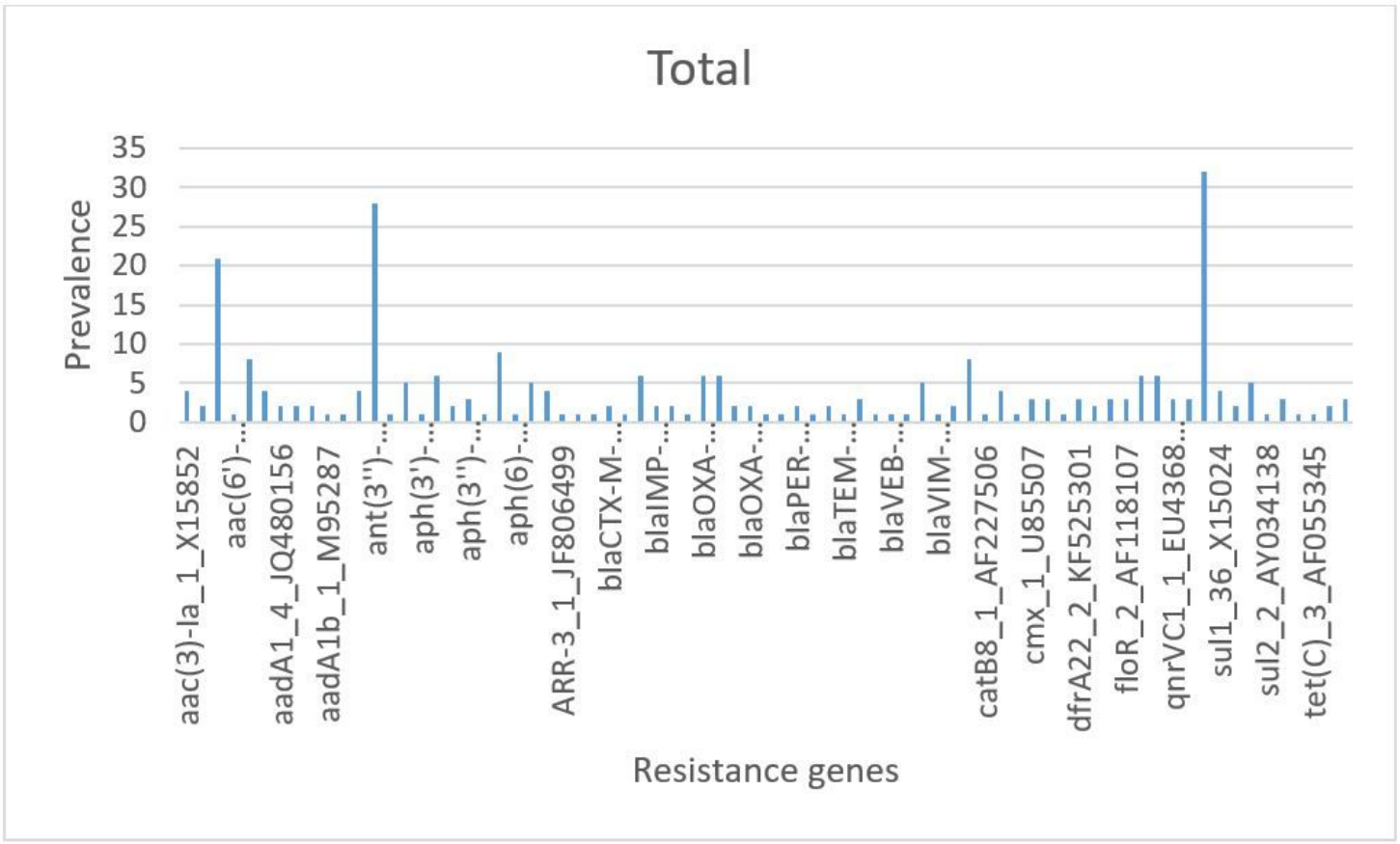

Figure 1

Prevalence of 74 AMR genes found in 40 Aeromonas plasmids after in silico analysis

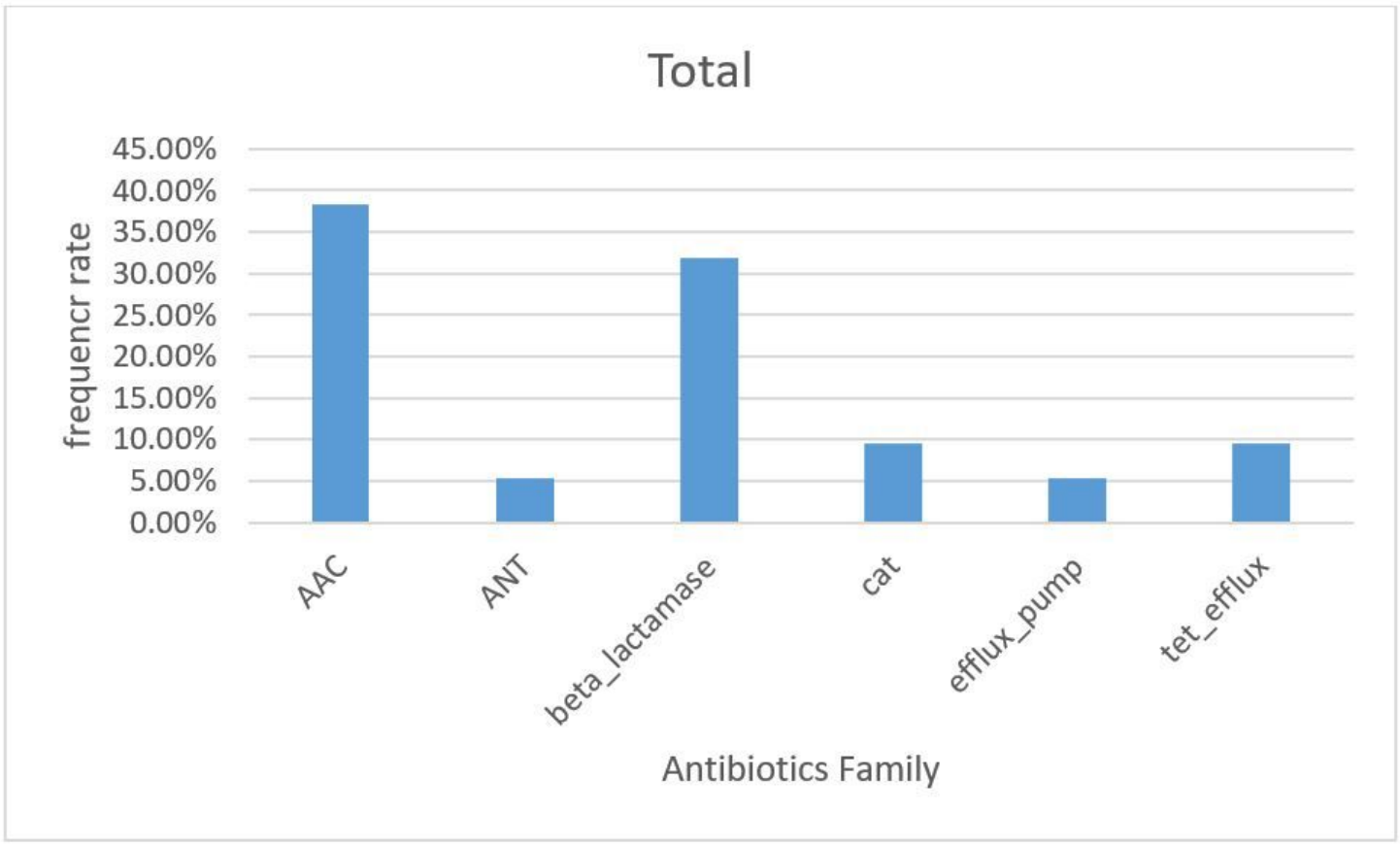

Figure 2

Frequency of Antibiotics family in AMS containing plasmids

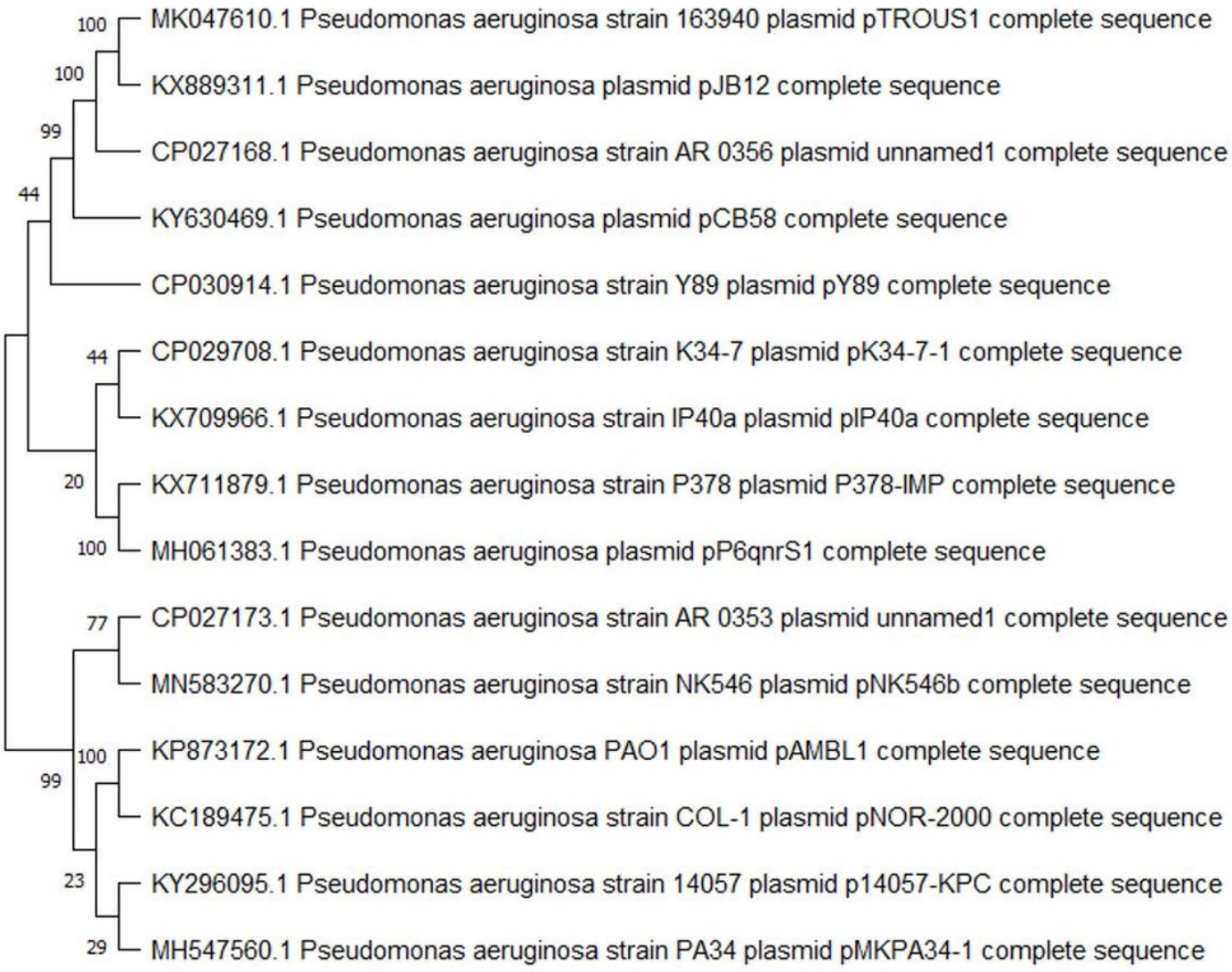

Figure 3

The phylogenetic analysis of 15 complete genome sequence of representative Pseudomonas aeruginosa plasmids by Maximum parsimony method using 1,000 bootstraps. The scale represents 0.1 substitutions per nucleotide position all of the accession numbers and full name of the strains were listed.