Kuwait J.Sci., Vol.50, No.(1A),January.2023,pp(1-15)

Insilico analysis on the complex relationship among antibiotic resistance, virulence genes and insertion sequences in *Pseudomonas aeruginosa*

Santhiya Kalimuthu[1, *]
[1]*Dept. of Biotechnology (FoE), Karpagam Academy of Higher Education, Coimbatore, 641021, Tamilnadu*

** Corresponding author: santhiya.k@kahedu.edu.in*

Abstract

Pseudomonas aeruginosa is the most frequent nosocomial pathogen, causing many infections in people and posing significant health risks worldwide. The current work attempts to understand the connection between antibiotic resistance genes (ARG), virulence factors (VF), and insertion sequences (IS) in *P. aeruginosa*. Fifty-six *P. aeruginosa* complete plasmids were retrieved from the NCBI database for this investigation. The CARD and Resfinder tools are used to discover ARG in *P. aeruginosa*. The VF analyzer and ISsaga tools are used to identify virulence genes and insertion sequences in the sorted plasmids. Using the tool PHASTER, the participation of prophage and integrase genes was discovered. Resistance to sulfonamide and beta-lactam was the most common ARG among the plasmids. Fil, pil, and XCP secretion systems are prevalent virulence genes. The prophage, integrase, and transposons were also identified. The correlation analysis of ARG, VF, and IS revealed that ISs, rather than virulence factors, had the most significant effect on the *P. aeruginosa* genome studied. As a result, an understanding of infectious bacterial profiles regarding pathogenicity islands and mobile elements is required to gain knowledge of their distribution and limit their spread throughout the world.

Keywords: Antibiotic resistance; health care; insertion sequence; mobile elements; virulence genes.

1. Introduction

Antibiotic resistance is a significant global burden due to increased infection caused by MDR gram-negative bacteria in recent years. It is estimated that nearly 0.7 million people die due to antibiotic-resistant infections (Dixit *et al.*, 2019). ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species*) encompasses six primary nosocomial-causing pathogens and its virulence increases day by day.

Pseudomonas aeruginosa, a gram-negative bacillus commonly found in the environment, especially in water resources, was first isolated from green pus in 1882 by Gossard. It has a massive set of regulatory genes in its genome (Ali *et al*., 2021). It has the unique feature of being able to survive under various environmental conditions, making it sustainable in the clinical environment (Jose *et al.*, 2017). The microorganism's pathogenicity depends on its strong affinity

for the host system. This interaction is mediated by a set of specialized factors called the Virulence Factor (VF); it is a significant measure of pathogenicity causing infection in the host system. *P. aeruginosa* strains have been found resistant to most current antibiotics present on the market, including beta-lactams, aminoglycosides, fluoroquinolones, and tetracyclines. A mobile element such as a transposon or insertion sequence plays a prominent role in transferring resistance genes among different bacterial species. The contribution of mobile elements in promoting bacterial resistance and genome evolution is very significant (Ho *et al*., 2009).

In this study, complete plasmid sequences of *P. aeruginosa* deposited in the National Centre for Biotechnology Information (NCBI) were utilized to detect the presence of antibiotic resistance genes and virulence factors. Additionally, insertion sequence analysis was also performed to decode the correlation between resistant genes and mobile elements in the pathogenicity of *P. aeruginosa*.

2. Material and Methods

2.1 Retrieval of complete plasmid sequence

The 56 complete plasmid sequences of *P. aeruginosa* (https://www.ncbi.nlm.nih.gov/genome/browse/#!/plasmids/Pseudomonas%20aeruginosa) were used for this in silico analysis. The sequences of *P. aeruginosa* were retrieved from the NCBI public database in February 2020.

2.2 Detection of antibiotic resistant genes

A Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca) and Resfinder (https://cge.cbs.dtu.dk/services/ResFinder/) are bioinformatics curated online resource tools providing a molecular basis for bacterial antimicrobial resistance (AMR) for nucleotide and protein sequences (Alcock *et al.*, 2020). The plasmids which harbor antibiotic-resistant genes were only taken for further analysis.

2.3 Detection of virulence gene

The presence of virulence genes for the sorted plasmids was found using the tool, Virulence Factor Database (VFanalyzer, http://www.mgc.ac.cn/VFs/); it is a comparative pathogenomics pipeline. It constructs conserved regions within the query genome and conducts iterative sequence similarity searches to avoid false positives (Liu *et al.*, 2019).

2.4 Insertion sequence analysis

The mobile elements like Insertion Sequence and Transposons in the sorted plasmids were analyzed using the ISsaga database (http,//issaga.biotoul.fr/issaga_index.php), which works under the platform of the ISfinder (Varani *et al.*, 2011).

2.5 Detection of prophage, integrase, and transposons

The presence of the prophage and integrase gene in the chosen plasmid was analyzed using the tool PHASTER (https://phaster.ca/) followed by BLAST. The transposons were found using the tool ISsaga database. The analysis was done only for the plasmids which have a correlation profile (ARG, IS and VF).

2.6 Phylogenetic analysis

The evolutionary relationship of plasmids was determined using MAFFT software (https://mafft.cbrc.jp/alignment/server/) for the chosen plasmids. MAFFT is based on the Fourier transform theory. It provides multiple sequence analysis and a phylogenetic interface to the user under one platform (Katoh *et al.*, 2019).

3. Result and Discussion

The genome analysis was conducted by analyzing 56 complete plasmid sequences (Table 1) of *P. aeruginosa* (1 kb to 500 kb) retrieved from the NCBI database. This study is exclusively based on the in silico approach. Out of 56 plasmids, 26 plasmid sequences were found to have various antibiotic resistance genes based on the annotation using the CARD and ResFinder tools, whereas 22 plasmids were identified to have 100% perfect resistance gene sequences in their genome.

Table 1. Fifty-six plasmids taken for the analysis

Accession Number	Strain	Size(Mb)	GC%	CDS	Release Date
CP011370.1	S04 90	0.159187	57.7321	175	2015-05-08T
CM003767.1	BH6	0.003652	55.8598	2	2016-02-08T
CP016215.1	PA121617	0.423017	56.4067	460	2016-07-07T
AJ877225.1	Nil	0.057121	59.4702	51	2005-06-01T
AM261760.1	Nil	0.089147	59.0059	97	2006-09-16T
AM778842.1	Nil	0.024179	63.8116	29	2007-08-21T
EU410482.1	Nil	0.00214	45.7944	1	2008-05-21T
HM560971.1	Nil	0.123322	60.5829	137	2014-10-15T
AY257538.1	Nil	0.103532	60.928	105	2012-11-04T
KC189475.1	Nil	0.02188	62.8108	23	2013-03-07T
KC609322.1	Nil	0.007995	55.5222	8	2013-09-12T
KC609323.1	Nil	0.031529	60.189	26	2013-09-14T
CM007350.1	PA3448	0.049094	58.8972	58	2016-10-27T
CP015000.1	PA7790	0.049021	58.8972	57	2016-11-08T
CP018048.1	DN1	0.317349	56.9376	384	2016-12-20T
CP020602.1	E6130952	0.036454	61.3348	40	2017-04-13T

CP017294.1	PA83	0.398087	59.3712	425	2017-07-08T
CP025052.1	PB353	0.059923	57.3386	75	2017-12-08T
CP025054.1	PB354	0.059923	57.3386	75	2017-12-08T
LT969519.1	RW109	0.555265	58.0933	588	2017-12-20T
LT969521.1	RW109	0.151612	57.2778	162	2017-12-20T
CP027173.1	AR_0353	0.041559	60.7979	46	2018-03-13T
CP027175.1	AR_0230	0.071782	59.6877	82	2018-03-13T
CP027176.1	AR_0230	0.00135	48	2	2018-03-13T
CP027167.1	AR_0356	0.165365	55.7857	200	2018-03-13T
CP027168.1	AR_0356	0.057053	60.8592	68	2018-03-13T
CP027170.1	AR_0356	0.438531	57.1435	509	2018-03-13T
CP029091.1	AR441	0.165365	55.7857	190	2018-05-02T
CP029092.1	AR441	0.057052	60.8585	67	2018-05-02T
CP029094.1	AR441	0.438529	57.1426	490	2018-05-02T
CP029095.1	AR439	0.001129	54.3844	2	2018-05-02T
CP029096.1	AR439	0.437392	56.8778	474	2018-05-02T
CP020561.2	CR1	0.046804	59.1894	56	2018-04-03T
CP029708.1	K34-7	0.00444	30.0676	3	2018-06-16T
CP030914.1	Y89	0.085842	60.0953	95	2018-08-14T
CP032256.1	AR_0111	0.129422	57.515	150	2018-09-19T
CP029714.1	BH9	0.041024	63.4945	26	2018-11-19T
CP033772.1	FDAARGOS_532	0.001249	51.7214	1	2018-11-26T
CP033773.1	FDAARGOS_532	0.001089	46.3728	1	2018-11-26T
CP033834.1	FDAARGOS_570	0.036032	61.2816	42	2018-11-26T
CP034355.1	IMP-13	0.130306	57.652	147	2018-12-19T
LS998784.1	1	0.024853	57.0635	31	2019-01-29T
CP039294.1	PABL048	0.414954	56.5819	483	2019-04-22T
CP040126.1	PA298	0.395774	56.8643	454	2019-05-20T
CP041355.1	AZPAE15042	0.185168	59.3385	205	2019-07-18T
CP041355.1	C79	0.04018	58.0762	37	2019-08-06T
CM017760.1	TC4411	0.419683	56.9659	478	2019-08-23T
CP042268.1	HOU1	0.167069	64.9259	165	2019-09-15T
CP043482.1	GIMC5001:PAT-23	0.049805	59.3796	61	2019-09-15T
CP043548.1	GIMC5002:PAT-169	0.049805	59.3896	59	2019-09-15T
CP024631.1	PA59	0.046627	59.1331	54	2019-11-05T
CP039989.1	T2436	0.422811	56.8755	472	2019-12-01T
CP039991.1	T2101	0.439744	56.9816	492	2019-12-01T
CM019124.1	4068	0.051059	59.5683	62	2019-12-05T
CP049162.1	MS14403	0.05013	59.601	67	2020-03-02T
KC543497.1	PA96	0.500839	57.598	545	2013-09-12T

The antibiotic-resistant gene analysis of twenty-two *P. aeruginosa* plasmids (Table 2) indicates that the strains harbored genes mediating resistance to antimicrobial-resistant groups, namely aminoglycosides, beta-lactams, fluoroquinolone, macrolide-lincosamide-streptogramin B (MLS), phenicol, sulfonamide and tetracycline. The AMR genes against sul were found to be prominent (n=12), followed by those against OXA (n=7) and KPC (n=4 each). Most of the plasmids encode sulfonamide and beta-lactam resistance (Figure. 1).

Table 2. Resistance genes detected in the 22 plasmids of *P. aeruginosa*

Accession No	ARO Term	AMR Family	Drug Class	Resistance Mechanism
CP011370.1	VIM-2	VIM	carbapenem, cephalosporin, cephamycin, penam	antibiotic inactivation
	aac(6')-29b	AAC(6')	aminoglycoside antibiotic	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
CM003767.1	KPC-2	KPC	monobactam, carbapenem, cephalosporin, penam	antibiotic inactivation
	Arma	16S rRNAmethyltransferase	aminoglycoside antibiotic	antibiotic target alteration
	IMP-45	IMP	carbapenem, cephalosporin, cephamycin, penam	antibiotic inactivation
	OXA-1	OXA	cephalosporin, penam	antibiotic inactivation
CP016215.1	catB3	chloramphenicol acetyltransferase (CAT)	phenicol antibiotic	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
	QnrVC6	quinolone resistance protein	fluoroquinolone antibiotic	antibiotic target protection
	mph	macrolide phosphotransferase (MPH)	macrolide antibiotic	antibiotic inactivation
	APH(3')-Ia	APH(3')	aminoglycoside antibiotic	antibiotic inactivation
AJ877225.1	aac(3)-I	AAC(3)	aminoglycoside antibiotic	antibiotic inactivation
	aadA5	ANT(3")	aminoglycoside antibiotic	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
AM261760.1	TEM-2	TEM	monobactam, cephalosporin, penam,	antibiotic inactivation
	aadA1	ANT(3")	aminoglycoside antibiotic	antibiotic inactivation

	tet(A)	major facilitator superfamily (MFS)	tetracycline antibiotic	antibiotic efflux
	dfrA1	trimethoprim resistant dihydrofolatereductase	diaminopyrimidine antibiotic	antibiotic target replacement
	SAT-2	streptothricinacetyltransferase (SAT)	nucleoside antibiotic	antibiotic inactivation
KC189475.1	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
	VIM-2	VIM	carbapenem, cephalosporin, cephamycin, penam, monobactam,	antibiotic inactivation
KC609323.1	KPC-2	KPC	carbapenem, cephalosporin, penam	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
	aac(6')-Ib	AAC(6')	aminoglycoside antibiotic	antibiotic inactivation
CP025052.1	OXA-101	OXA	cephalosporin, penam	antibiotic inactivation
	TEM-1B	TEM	monobactam, cephalosporin, penam	antibiotic inactivation
	CTX-M-30	CTX-M	cephalosporin	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
CP025054.1	aac(6')-Ib	AAC(6')	aminoglycoside antibiotic	antibiotic inactivation
	TEM-1B	TEM	monobactam, cephalosporin, penam	antibiotic inactivation
	CTX-M-30	CTX-M	cephalosporin	antibiotic inactivation
CP027173.1	GE'S-1	GES	carbapenem, cephalosporin, penam	antibiotic inactivation
CP027168.1	KPC-2	KPC	monobactam, carbapenem, cephalosporin	antibiotic inactivation
CP029092.1	KPC-2	KPC	monobactam, carbapenem, cephalosporin,	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
CP029096.1	IMP-18	IMP	carbapenem, cephalosporin, cephamycin, penam	antibiotic inactivation
	each	small multidrug resistance (SMR) antibiotic efflux pump	fluoroquinolone antibiotic	antibiotic efflux
	AAC(6')-Il	AAC(6')	aminoglycoside antibiotic	antibiotic inactivation
CP02970	tet(K)	major facilitator	tetracycline antibiotic	antibiotic efflux

8.1		superfamily (MFS) antibiotic efflux pump		
	VIM-6	VIM	carbapenem, cephalosporin, cephamycin, penam	antibiotic inactivation
	OXA 10	OXA	cephalosporin, penam	antibiotic inactivation
CP03383 4.1	aac(6')-Ib3	AAC(6')	aminoglycoside antibiotic	antibiotic inactivation
	aph(6)-Id	APH(6)	aminoglycoside antibiotic	antibiotic inactivation
	cmx	major facilitator superfamily (MFS) antibiotic efflux pump	phenicol antibiotic	antibiotic efflux
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
CP03929 4.1	aadA10	ANT(3")	aminoglycoside antibiotic	antibiotic inactivation
	OXA-10	OXA	cephalosporin, penam	antibiotic inactivation
	ANT(2")-Ia	ANT(2")	aminoglycoside antibiotic	antibiotic inactivation
	IMP-45	IMP	carbapenem, cephalosporin, cephamycin, penam	antibiotic inactivation
CP04012 6.1	catB3	chloramphenicol acetyltransferase (CAT)	phenicol antibiotic	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
CP04135 5.1	VIM-1	VIM	carbapenem, cephalosporin, cephamycin, penam	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
CP04226 8.1	CpxR	resistance-nodulation-cell division (RND) antibiotic efflux pump	a macrolide antibiotic	antibiotic efflux
	APH(3")-Ib	APH(3")	aminoglycoside antibiotic	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
	OXA-10	OXA	cephalosporin, penam	antibiotic inactivation
CP03998 9.1	cmlA5	major facilitator superfamily (MFS) antibiotic efflux pump	phenicol antibiotic	antibiotic efflux
	arr-2	rifampin ADP-ribosyltransferase (Arr)	rifamycin antibiotic	antibiotic inactivation
	ANT(2")-Ia	ANT(2")	aminoglycoside antibiotic	antibiotic inactivation
	VEB-2	VEB	monobactam,	antibiotic

			cephalosporin	inactivation
	OXA-10	OXA	cephalosporin, penam	antibiotic inactivation
	FEB-1	VEB	monobactam, cephalosporin	antibiotic inactivation
	tet(G)	major facilitator superfamily (MFS)	tetracycline antibiotic	antibiotic efflux
CP039991.1	ANT(2'')-Ia	ANT(2'')	aminoglycoside antibiotic	antibiotic inactivation
	CARB-2	CARB	Penam	antibiotic inactivation
	FEB-1	VEB	monobactam, cephalosporin	antibiotic inactivation
	sul1	sul	cephalosporin, penam	antibiotic inactivation
	OXA-10	OXA	cephalosporin, penam	antibiotic inactivation
	sul1	sul	sulfonamide antibiotic	antibiotic target replacement
KC543497.1	AAC(6')-Ib4	AAC(6')	aminoglycoside antibiotic	antibiotic inactivation
	IMP-9	IMP	carbapenem, cephalosporin, cephamycin, penam, penem	antibiotic inactivation

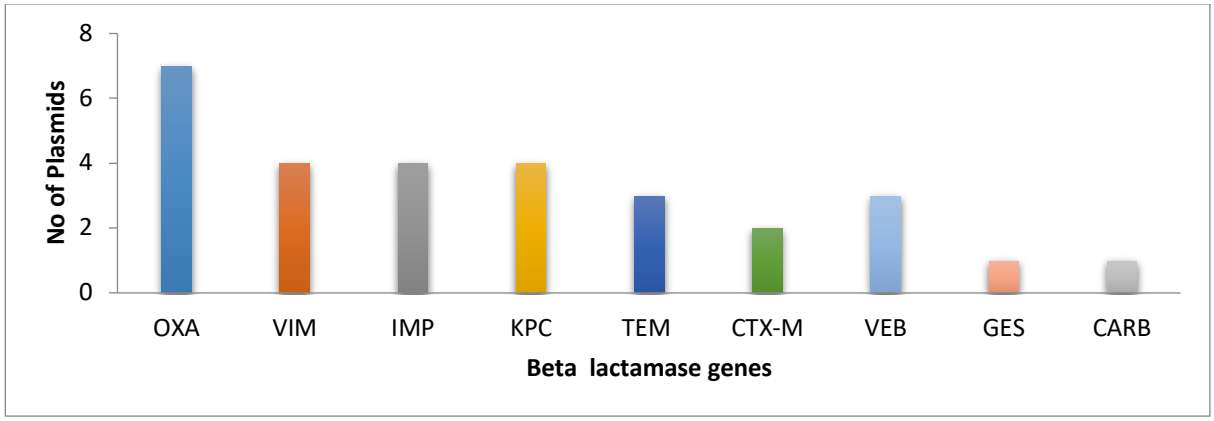

Fig. 1. Frequency of Beta-lactam resistant genes in the plasmids studied

Sulfonamide resistance is frequently associated with florfenicol and oxytetracycline resistance, and it will be transferred to-resistant genes through selection (Dominguez *et al*., 2019). However, in this study, sul genes coexisted with beta-lactam resistant genes like OXA10, CTX-M-30, and TEM in most of the plasmids. The beta-lactam resistant genes like OXA, VIM, IMP, TEM, CTX, and KPC have been detected in the plasmids taken for the analysis. Beta-lactam antibiotics are commonly used for the treatment of *P. aeruginosa* infections. Antibiotic resistance is caused by lactamase enzymes cleaving antibiotics (Zilberberg *et al*., 2010). In *P. aeruginosa*, there is a significant association between toxin secretion and beta-lactamase

producers. In clinical isolates of *P. aeruginosa*, pigment production was more strongly linked to beta-lactam antibiotic resistance than the development of virulence components such as elastases and proteases (Finlayson *et al*., 2011).

3.1 Involvement of Virulence Factors

Among 22 plasmids analyzed in the VFDB database, only 11 plasmids were found to have virulence genes in their genome (Table 3). The virulence factors for adherence and secretion systems were prominent in the *P.aeruginosa* plasmid taken for the study.

Table 3. Virulence gene profile for plasmids

S. No	Accession No	Virulence genes
1	CP039294.1	xcpU
2	CP016215.1	mtrD, xcpU, xcpA
3	AJ877225.1	cyaB, cyaA
4	CP029096.1	bplL, xcpU, xcpA
5	CP039989.1	xcpU, xcpA
6	CP011370.1	pilB, pilQ
7	CP039991.1	xcpU, xcpA
8	CP040126.1	xcpU, xcpA
9	CP027173.1	fliD
10	KC543497.1	xcpU, xcpA
11	CP042268.1	fimV, xcpQ, xcpR, xcpS, xcpY, xcpW, xcpP, xcpU, xcpT, xcpV, pilB, pilT, pilQ, pilB, gspE, bplL, tagT, ybtP, eccCa1, cyaB, fha1, pchl, fleR, popN

P.aeruginosa has various cell surface pili and flagella related to genes responsible for the twitching motility of the bacteria and involved in its biogenesis, expression, and functionality of pilus. It attaches to the host cell and guides the bacteria to accelerate along the cell surface. It also helps biofilm formation, avoids the host immune system, and creates resistance against antibiotics (Sriramulu *et al.*, 2005).The secretory system plays a significant role in bacterial pathogenicity. There were about eight types of secretory systems (Tseng *et al.*, 2009). Xcp factor comes under T2SS and has been identified in *P. aeruginosa*. Xcp needs the proper localization of PilB and PilC and secretes toxins in the extracellular fluid. Xcp secretes the quorum sensing regulated virulence factors elastaseA, elastase B and exotoxin A.

3.2 Existence of Insertion sequence in plasmids

A mobile genetic element such as transposons, insertion sequence, and integrons plays a crucial role in *P. aeruginosa* to become multidrug-resistant bacteria. The sorted plasmids are predicted to have 12 different transposon families and various insertion sequences like ISKpn6, ISKpn7,

ISPa16, ISPa17, IS6100, ISPt3, ISPen2, TnAs3 and IS222. Among the 12 types, IS21 and IS6 are the most prominent families (Figure. 2).

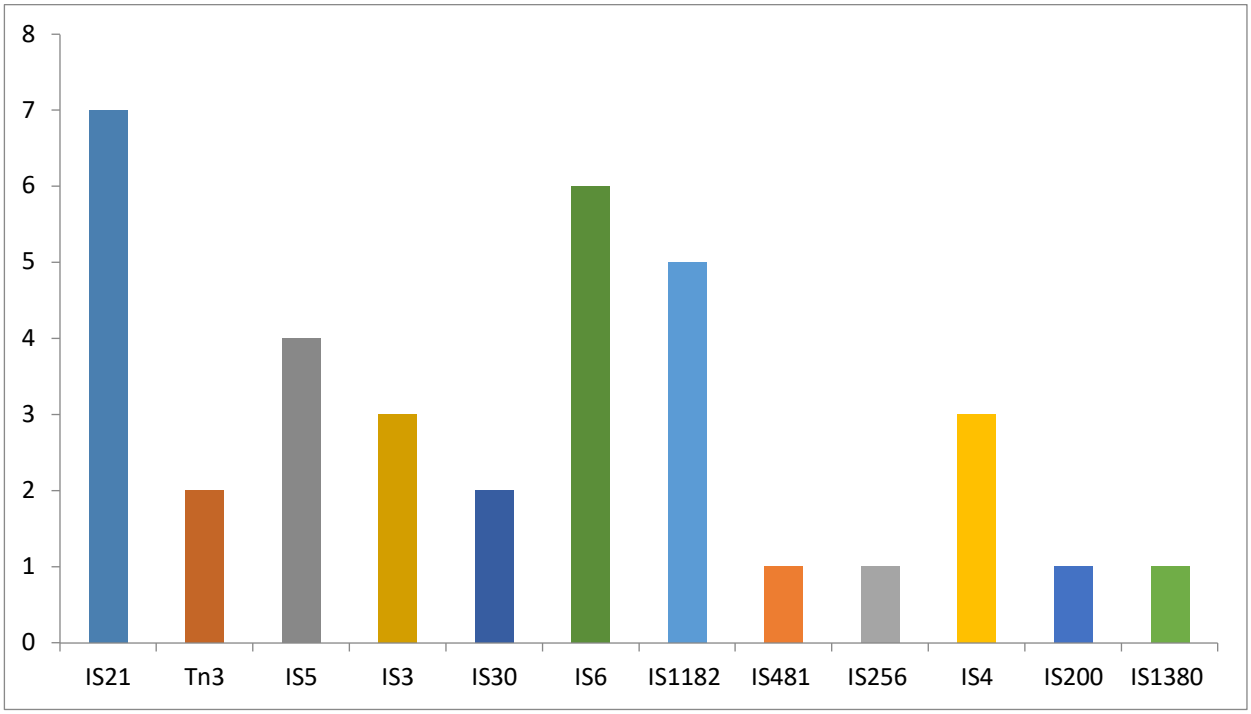

Fig. 2. ISs profile for plasmids taken for study

The study by Bouteille *et al., 2004* suggested that IS mediated efflux pump depression has been detected in the IS21 family due to more repressors, especially in *P. aeruginosa*, that tend to increase the occurrence of beta-lactam resistant genes in the genome. According to the study, the majority of the beta-lactam resistance identified has an IS21 family of ISs.

The resistant gene KPC belongs to the beta-lactam resistance and has been chiefly carried along with the Tn3 like transposon family. This genome arrangement plays a role in disseminating the blaKPC gene among varied species, especially in *P. aeruginosa*. The current study shows the presence of the blaKPC gene along with ISKpn6 and ISKpn7 belonging to the IS1182 and IS21 families, respectively. The study by Johnson TA *et al*., 2016 revealed that IS6100 type transposon has an abundance of genes conferring resistance to about six antibiotic classes and class 1 integrase. In relevance to this study, IS6100 is likely to have a resistance gene profile (APH(3")-Ib, sul1, cmlA5, arr-2, ANT (2")-Ia, VEB-2) in two plasmids and another resistance pattern (OXA-10, sul1, AAC(6')-Ib4, IMP-9) found in three plasmids.

The correlation profile among ARG, IS, and VG for 7 out of 22 *P.aeruginosa* plasmids taken for analysis is listed in Table 4. The correlation analysis shows that five plasmids had similar kinds of ARGs and are profiled in their genomes, and it is interesting that VF was not found in those five plasmids. The remaining two plasmids found virulence genes for adherence with similar ARG patterns.

Table 4. Correlation profile on ARG, IS and VF

S. No	Accession No	Sample source	Location	ARG	IS	VF
1	KC609323.1	Bronchial secretion	France	KPC-2	ISApu1,ISKpn6	No
2	CP025052.1	Urine	USA	sul1 TEM-1 CTX-M-30	IS6100,ISEcp1	No
3	CP025054.1	Urine	USA	sul1 TEM-1 CTX-M-30	IS6100	No
4	CP027168.1	-	USA	KPC-2	ISKpn6, ISKpn7	No
5	CP029092.1	-	USA	KPC-2	ISKpn6, ISKpn7	No
6	CP039989.1	Adult male sputum	UK	APH(3")-Ib sul1 OXA - 10 arr-2 ANT(2")-Ia	IS10A, IS6100	xcpU, xcpA
7	CP039991.1	Adult male sputum	UK	VEB-2 OXA-10 FEB-1 ANT(2")-Ia FEB-1 sul1	IS6100	xcpU, xcpA

3.3 Detection of Prophage, Integrase and Transposons

Prophages are bacteriophages that have been identified to contribute to interstrain genetic diversity by integrating into bacterial chromosomes via an integrase gene. A transposase is indeed an attributed recombinase that facilitates transposition or an integrase in the case of a retrovirus with RNA intermediate in its life cycle. The transposons Tn3, ISEc9, IS600, IS3, IS6100, Tn5041, and IS1479 were identified in the plasmids taken for the study using the ISsaga database. The same resistance gene appears to be linked to many mobile elements; a closer look shows a more complicated picture.

The prophage and integrase genes were found using the PHASTER tool. The intI1 integrase was present in three plasmids taken for the study. Integrons can significantly improve the host bacterium's fitness in clinical settings. Several outbreaks of bacteria generating beta-lactamases have been linked to integron-associated antibiotic resistance genes. The presence of intI1 in recycled water is strongly linked with sul1 and tet antibiotic-resistant genes (Gillings *et al*., 2015).

Table 5. Plasmids bearing Integrase, Prophage and Transposon

S. No	A. No	Integrase	Prophage	Transposon
1	CP029096.1	intI1	exonuclease, Hef nuclease, methyltransferase	Transposase IS3/IS911family
2	CP025054.1	intI1	No	ISEc9 and IS6100
3	CP025052.1	intI1	No	ISEc9 and IS6100
4	CP039989.1	No	exonuclease, Hef nuclease	Tn3
5	CP039991.1	No	exonuclease, Hef nuclease, methyltransferase,	Tn3 and IS600
6	KC609323.1	No	No	Tn5041 and Tn5044
7	CP027168.1	No	No	Tn5041 and Tn5044

Prophages detected in this study are exonuclease, Hefnuclease and methyltransferase (Table 5). Many bacteriophages carry methyltransferase genes. Methylation seems to be required for pathogen survival in certain strains. Hef includes a domain linked to a wide range of endonucleases. However, it has no sequence homology with the other Hef families. The survival of a hef in a population depends on repeated transposition to various destinations followed by subsequent dissemination among phages via homing (Zund *et al*., 2021).

3.4 Phylogenetic analysis

Phylogenetic analysis was performed using MAFFT software. The analysis was done for seven plasmids with a correlation profile (ARG, IS, and VF) as per the above results. The result shows that the plasmids encoding the similar pattern of ARG and IS was presented under a single clade compared to other plasmids. The influence of prophage and integrase genes was not as prominent as per this phylogeny analysis. It indicates that plasmids play an essential role in promoting the transmission of ARG and mobile elements amongst varied plasmid categories and distant evolutionary lineages.

The sequences CP039989 and CP039991 have the same prophage and antibiotic resistance genes. However, the sequence KC609323 from France lacks any prophage genes, although all three plasmids belong to the same clade (Figure. 3). A similar situation happened in the sequences, CP027168 and CP029092.

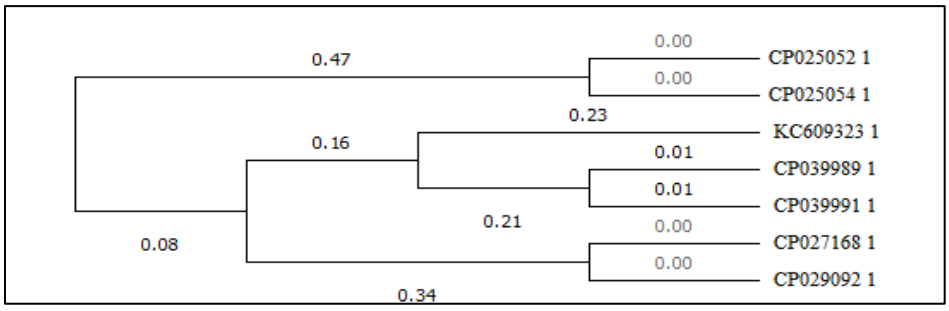

Fig. 3. Phylogeny relation of plasmids

Zhang *et al.* 2019 illustrated that the bacteria have different ARG profiles in different time intervals. However, selective pressure has little effect on most virulence genes. In this study, the absence of virulence factors was seen in some plasmids even though they tended to have ARG and ISs in their genome. This may be due to the genome reduction in the infectious bacteria, which tends to the absence of virulence factors (Beceiro *et al.*, 2013). Hence, the results suggest that the possible spread of *P. aeruginosa* among various microbial communities is due to the influence of some virulence genes. The abundance of insertion sequences in their genome plays a notable role in pathogenicity.

4. Conclusion

In this study, 22 out of 56 complete plasmids of *P. aeruginosa* were found to have known antibiotic resistance genes. The most prominent resistant genes are sulfonamide and beta-lactam and mainly related to adherence and xcp secretion system. IS21 and IS6 are the prominent IS families observed in plasmids taken for the analysis. The plasmids were identified as the intI1 gene and prophages like exonuclease, Hef nuclease, and methyltransferase. The correlation among ARG, VF, and IS indicates that IS elements occurring within the plasmids significantly influence the antibiotic resistance profile in the analysed *P. aeruginosa* plasmids. In contrast, the proportion of VF was lower and predicted to have less influence on the genome. It could be that environmental factors might have some influence on the genome features of ARGs and VFs in the plasmids taken for the study. However, the coexistence of antibiotic resistance genes and mobile elements needs to be considered to understand the dissemination and influence on each other in multidrug-resistant bacteria like *P. aeruginosa* to identify more specific and accurate drug treatments to overcome the infections in the future.

References

Alcock, B.P., Raphenya, A.R., Lau, T.T.Y., Tsang, K.K., Bouchard, M., Edalatmand, A., *et al*. (2020) CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Research, 48(**D1**): D517-D525.

Ali, F., Kamal, S., Shakeela, Q. & Ahmed, S. (2021) Extended-spectrum and Metallo-beta lactamase enzymes mediated resistance in Pseudomonas aeruginosa in clinically isolated specimens. Kuwait Journal of Science, 48(**2**): 1-9.

Beceiro, A., Tomás, M. & Bou, G. (2013) Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clinical Microbiology Reviews, 26(**2**):185-230.

Boutoille, D., Corvec, S., Caroff, N., Giraudeau, C., Espaze, E., Caillon, J., Plésiat, P., & Reynaud, A. (2004) Detection of an IS21 insertion sequence in the mexR gene of Pseudomonas aeruginosa increasing beta-lactam resistance. FEMS microbiology letters, 230(**1**), 143–146.

Dixit, A., Kumar, N., Kumar, S. &Trigun, V. (2019) Antimicrobial Resistance: Progress in the Decade since Emergence of New Delhi Metallo-β-Lactamase in India. Indian Journal of Community Medicine, 44(**1**):4-8.

Domínguez, M., Miranda, C. D., Fuentes, O., de la Fuente, M., Godoy, F. A., Bello-Toledo, H., & González-Rocha, G. (2019) Occurrence of Transferable Integrons and *sul* and *dfr* Genes Among Sulfonamide-and/or Trimethoprim-Resistant Bacteria Isolated From Chilean Salmonid Farms. Frontiers in microbiology, 10, 748.

Finlayson, E.A. & Brown, P.D, (2011) Comparison of antibiotic resistance and virulence factors in pigmented and non-pigmented *Pseudomonas aeruginosa*. The West Indian medical journal, 60 (**1**), 24–32.

Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M, & Zhu, Y.G. (2015). Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. ISME J, 9(**6**):1269-1279.

Ho Sui, S.J., Fedynak, A., Hsiao, W.W., Langille, M.G., & Brinkman, F.S. (2009) The association of virulence factors with genomic islands. PloS one, *4*(**12**), e8094.

Johnson, T. A., Stedtfeld, R. D., Wang, Q., Cole, J. R., Hashsham, S. A., Looft, T., Zhu, Y. G., &Tiedje, J. M. (2016) Clusters of Antibiotic Resistance Genes Enriched Together Stay Together in Swine Agriculture. mBio, 7(**2**), e02214–e2215.

Jose, J., Santhiya, K., Jayanthi, S. &Ananthasubramanian, M. (2017) Insertion sequence-based analysis of clinical isolates with NDM *(bla*NDM-1) resistance, Indian Journal of Biotechnology; 16(**2**): 182-188.

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20:1160-1166

Liu, B., Zheng, D.D., Jin, Q., Chen, L.H. & Yang, J. (2019) VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res; 47(D1): D687-D692.

Sriramulu, D.D., Lunsdorf, H., Lam, J.S. & Romling, U. (2005) Microcolony formation: a novel biofilm model of Pseudomonas aeruginosa for the cystic fibrosis lung. Journal of Medical Microbiol;54:667-676.

Tseng, T.T., Tyler, B.M. & Setubal, J.C. (2009) Protein secretion systems in bacterial-host associations, and their description in the Gene Ontology. BMC Microbiology, 9, S2.

Varani, A.M., Siguier, P., Gourbeyre, E., Charneau, V. & Chandler, M. (2011)ISsaga is an ensemble of web-based methods for high throughput identification and semi-automatic annotation of insertion sequences in prokaryotic genomes. Genome Biology, 12(**3**): R30.

Zilberberg, M.D., Chen, J., Mody, S.H., Ramsey, A.M. &Shorr, A.F. (2010) Imipenem resistance of Pseudomonas in pneumonia: a systematic literature review. BMC Pulmonary Medicine, 10:45.

Zünd, M., Ruscheweyh, H.J., Field, C.M., Natalie, M., Miguelangel, C., Daniel, H. et al. (2021) High throughput sequencing provides exact genomic locations of inducible prophages and accurate phage-to-host ratios in gut microbial strains. Microbiome, 9, 77.

Submitted: 06/06/2021
Revised: 17/05/2022
Accepted: 01/06/2022
DOI: 10.48129/kjs.14591