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

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Software article

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Abstract

Introduction: The aim of this study is 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 population 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 – 55265 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%.
| 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    | 57053   | 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 PA01 plasmid pAMBL2 | 60.4    | 24133    | yes |
| PA67          | Pseudomonas aeruginosa PA01 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.
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  | 2  | 10 |     |     |     | 0.2       |
| 6   | Pseudomonas aeruginosa plasmid p727-IMP            | 2  | 2  | 2   | 1  | 1  | 1  | 1   | 1   | 13  | 0.6       |
| 7   | Pseudomonas aeruginosa plasmid pA681-IMP           | 3  | 3  | 2   | 1  | 2  | 4  |     |     |     | 0.4       |
| 8   | Pseudomonas aeruginosa plasmid pBS228              | 1  | 1  |     | 1  | 1  | 4  |     |     |     | 0.266667 |
| 9   | Pseudomonas aeruginosa plasmid pCB58               | 10 | 1  | 1   | 1  | 1  | 13 |     |     |     | 0.266667 |
| 10  | Pseudomonas aeruginosa plasmid pJB12               | 5  | 1  | 1   | 1  | 1  | 8  |     |     |     | 0.266667 |
| 11  | Pseudomonas aeruginosa plasmid pJB35               | 5  | 1  | 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   | 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  | 1  |    |    |    |    | 5     | 0.266667       |
| 16  | Pseudomonas aeruginosa strain 163940 plasmid pTROUS1 | 1  | 2  | 1   | 1  | 1  |    |    |    |    | 5     | 0.266667       |
| 17  | Pseudomonas aeruginosa strain AR_0356 plasmid unnamed2 | 4  | 2  | 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  | 2  |    |    |    |    | 10    | 0.266667       |
| 20  | Pseudomonas aeruginosa strain CF39S plasmid pCF39S | 8  | 2  | 2   | 2  | 2  |    |    |    |    | 14    | 0.266667       |
| 21  | Pseudomonas aeruginosa strain D5170990 plasmid pD5170990 | 9  | 1  | 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  | 1  | 8  |    |    |    | 0.26667             |
| 24  | Pseudomonas aeruginosa strain HN39 plasmid pHN39-SIM | 1  | 2  | 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 |    |    | 0.33333            |
| 27  | Pseudomonas aeruginosa strain PA121617 plasmid pBM413 | 6  | 4  | 4   | 2  | 4  | 4  | 24 |    | 0.33333            |
| 28  | Pseudomonas aeruginosa strain PA298 plasmid pBM908  | 4  | 6  | 2   | 4  | 2  | 18 |    |    | 0.33333            |
| 29  | Pseudomonas aeruginosa strain PA34 plasmid pMKPA34-1 | 5  | 1  | 1   | 1  | 1  | 1  | 10 |    | 0.4                |
| 30  | Pseudomonas aeruginosa strain PA4T437 plasmid pOXA-198 | 4  | 1  | 1   | 1  | 7  |    |    |    | 0.26667            |
| 31  | Pseudomonas aeruginosa strain PAB546 plasmid pNK546-KPC | 1  | 1  | 1   | 1  | 4  |    |    |    | 0.26667            |
| 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 |    |    |    | 0.26667            |
| 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  |       | 0.26667       |
| 36  | Pseudomonas aeruginosa strain PB354 plasmid pPB354_1 | 2  | 3  | 2   | 1  |     |    |     |     | 8   |       | 0.26667       |
| 37  | Pseudomonas aeruginosa strain S04 90 plasmid S04_90  | 4  | 2  | 2   | 8  |     |    |     |     | 8   |       | 0.2           |
| 38  | Pseudomonas aeruginosa strain T2101 plasmid pBT2101  | 8  | 12 | 8   | 4  |     |    |     |     | 32  |       | 0.26667       |
| 39  | Pseudomonas aeruginosa strain T2436 plasmid pBT2436  | 10 | 4  | 2   | 2  | 4  | 2  |     |     | 24  |       | 0.4           |
| 40  | Pseudomonas aeruginosa strain TC411 TC411 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 acetyltransferases 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)

| 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 population 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.
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Authors' contributions

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

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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.