BRIEF REPORT

Genome-wide analyses reveal antibiotic resistance genes and mechanisms in pathogenic *Pseudomonas* bacteria [version 1; peer review: 1 approved, 1 not approved]

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Abstract

Background: The global emergence and re-emergence of antibiotic resistance among the *Pseudomonas* pathogens causes great problems to patients undergoing chemotherapy. However, there is limited comparative information on the antibiotic resistance genes (ARGs) and mechanisms across the *Pseudomonas* pathogenic groups.

Methods: The complete genomes of five *Pseudomonas* pathogen groups, *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. stutzeri* and *P. syringae*, were analyzed for ARGs.

Results: A significant number of ARGs were identified in the *P. aeruginosa* genome compared to the other *Pseudomonas* pathogens. The opportunistic pathogens *P. stutzeri* and *P. putida* were shown to be the closest to *P. aeruginosa* with an average nucleotide identity (%) of 80.30 and 79.52. The pathogen genome with the least hit was *P. stutzeri*. The four major antibiotic resistance mechanisms that include the efflux, inactivation, target alteration and efflux::target alteration were reported.

Conclusion: The findings of this brief report could be useful in understanding the chemotherapeutics against antibiotic resistance strains of *Pseudomonas* pathogens

Keywords

Pseudomonas, Antibiotics Resistance, bacterial pathogen, *P. aeruginosa*

Open Peer Review**Approval Status**

	1	2
version 1		
04 Aug 2020	view	view
1. Asad U. Khan , Aligarh Muslim University, Aligarh, India		
2. Paula Blanco , Universidad Complutense de Madrid, Madrid, Spain		

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Introduction

The emergence of Gram-negative bacterial antibiotics resistance is a growing threat to antibiotic therapy. The bacterial pathogens in the genus *Pseudomonas* are mostly opportunistic that cause great damage and loss of life (Breidenstein *et al.*, 2011; Evans *et al.*, 2008; Kawai, 1974; Lalucat *et al.*, 2006). These *Pseudomonas* pathogens are pervasive that is able to infect, survive and proliferate in a wide range of biotic and abiotic environments (Azam & Khan, 2019; Silby *et al.*, 2011). Presently, there are seven groups in the genus *Pseudomonas*, with *P. aeruginosa* being the most pathogenic (Barbier *et al.*, 2013) and which causes high morbidity and mortality in cystic fibrosis patients and immunocompromised individuals (Sadikot *et al.*, 2005). The other *Pseudomonas* pathogenic groups include *P. fluorescens* (Biaggini *et al.*, 2015), *P. putida* (Fernández *et al.*, 2015), *P. stutzeri* (Lalucat *et al.*, 2006) and *P. syringae* (Xin *et al.*, 2018). *P. aeruginosa* is the major cause of infections in developed countries due to its highly evolved resistance to a wide variety of antibiotics (Hancock & Speert, 2000) making it very difficult to treat and limiting therapeutics (Breidenstein *et al.*, 2011). Even though *P. aeruginosa* is the most studied, there are some other *Pseudomonas* species that exhibits opportunistic pathogenic behavior to animals (*P. fluorescens*, *P. putida* and *P. stutzeri*) (Azam & Khan, 2019) and plants (*P. syringae*) (Xin *et al.*, 2018). Most of the known antimicrobial resistance (AMR) gene and mechanistic studies are focused on *P. aeruginosa* with little attention directed to other *Pseudomonas* pathogens. Hence, there is need to investigate different *Pseudomonas* pathogens genomes for diverse antibiotic resistance genes (ARGs) and mechanism. Therefore, this brief report concisely revealed the ARGs, mechanisms and drugs in *P. aeruginosa* in comparison to other *Pseudomonas* pathogens.

Methods

The complete genomes of the five groups of *Pseudomonas* pathogens, which included the *P. aeruginosa* (NC_002516.2), *P. fluorescens* (NC_016830.1), *P. putida* (NC_002947.4), *P. stutzeri* (NC_015740.1) and *P. syringae* (NC_007005.1) fasta file sequences, were downloaded from The National Center for Biotechnology Information Genome database. The five *Pseudomonas* pathogen genomes were selected to represent the *Pseudomonas* groups. The fasta file format of the genome sequence of bacteria were thoroughly analyzed for ARGs

on the bulk analysis Resistance Gene Identifier (RGI) 5.1.0, CARD 3.0.9 Platform (Alcock *et al.*, 2020) to extract AMR Genes, AMR Gene Family, Drug Class and Resistance Mechanism data. Default select criteria, which identified gene based on strict or perfect only was used. On the RGI platform, each genome sequence file was uploaded and all settings were left at default. The resistance genes, mechanism and drugs obtained from RGI platform were further analyzed using Prism 8 for number of ARG hits, gene family, mechanism and drug class per *Pseudomonas* species.

Results

The five *Pseudomonas* pathogens show significant genome similarity (Table 1). *P. stutzeri* and *P. putida*, which have been reported to be opportunist pathogens to humans, were shown to be the closest relations to *P. aeruginosa*, with an average nucleotide identity (%) of 80.30 and 79.52. *P. syringae*, which infects plants, had the lowest average nucleotide identity (%) of 78.67. A number of antimicrobial resistance hits and genes were identified across the five *Pseudomonas* pathogens, but the highest hits were seen in the *P. aeruginosa* genome. The pathogen genome with the least hit was *P. stutzeri* (Figure 1A, B). The four major antibiotic resistance mechanisms identified were the efflux, inactivation, target alteration and efflux::target alterations in the *P. aeruginosa* genome. The efflux, inactivation and efflux::target alterations were identified in the *P. fluorescens* genome, while only the efflux and inactivation alterations were identified in the *P. putida*, *P. stutzeri* and *P. syringae* genomes (Figure 2A). The number of drug classes that *P. aeruginosa* was shown to be resistant to is also shown in Figure 2B.

Discussion

There is an increasing interest and focus in the antibiotic resistance in pathogenic *Pseudomonas* (Blair *et al.*, 2014; Blanco *et al.*, 2016; Li *et al.*, 2015; Soto, 2013; Webber & Piddock, 2003). Hence, this report took advantage of the available *Pseudomonas* pathogens genomes and analysed for antibiotic resistance genes and mechanisms against different available drugs.

The significant number of ARGs and mechanisms were identified in the genome of *P. aeruginosa*, which is more virulent and well-studied compared to other species. *P. aeruginosa* also infects a wide range of plants and animals, including humans

Table 1. *Pseudomonas* pathogen genomes used in this study.

S/N	<i>Pseudomonas</i> groups	Pathogenic	Genome accession No.	Average nucleotide identity (%)
1	<i>P. aeruginosa</i>	Pathogenic to plants and animals (Azam & Khan, 2019)	NC_002516.2	RG
2	<i>P. fluorescens</i>	opportunistic human pathogens (Biaggini <i>et al.</i> , 2015)	NC_016830.1	79.25
3	<i>P. putida</i>	opportunistic human pathogens (Fernández <i>et al.</i> , 2015)	NC_002947.4	79.52
4	<i>P. stutzeri</i>	opportunistic human pathogens (Lalucat <i>et al.</i> , 2006)	NC_015740.1	80.30
5	<i>P. syringae</i>	Pathogenic to plants (Xin <i>et al.</i> , 2018)	NC_007005.1	78.67

Figure 1. *P. aeruginosa* exhibited more Antibiotics Resistance Genes (ARGs). (A) The number of hit per each *Pseudomonas* pathogen genome (B). Number of ARGs per each *Pseudomonas* pathogen genome.

Figure 2. Antibiotic efflux resistance mechanism is widely spread across the *Pseudomonas* pathogen genome and highly abundant in *P. aeruginosa*. (A) Number of resistance mechanism per each *Pseudomonas* pathogen genome. (B) *P. aeruginosa* resistance drug class. Drug class key: A= aminoglycoside antibiotic; B= fluoroquinolone antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic; C= macrolide antibiotic, carbapenem, tetracycline antibiotic, acridine dye, diaminopyrimidine antibiotic, phenicol antibiotic; D= macrolide antibiotic, fluoroquinolone antibiotic, aminoglycoside antibiotic, carbapenem, cephalosporin, cephamycin, penam, tetracycline antibiotic, acridine dye, phenicol antibiotic; E= macrolide antibiotic, fluoroquinolone antibiotic, aminoglycoside antibiotic, cephalosporin, penam, tetracycline antibiotic, aminocoumarin antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic; F= macrolide antibiotic, fluoroquinolone antibiotic, cephalosporin, penam, tetracycline antibiotic, aminocoumarin antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic; G= macrolide antibiotic, fluoroquinolone antibiotic, monobactam, carbapenem, cephalosporin, cephamycin, penam, tetracycline antibiotic, peptide antibiotic, aminocoumarin antibiotic, diaminopyrimidine antibiotic, sulfonamide antibiotic, phenicol antibiotic, penem; H= peptide antibiotic; I = phenicol antibiotic; J = sulfonamide antibiotic.

(Azam & Khan, 2019; Hancock & Speert, 2000; Sadikot *et al.*, 2005). *P. aeruginosa* is of great medical importance due to its exhibition of multidrug resistance and its association with serious illnesses (Breidenstein *et al.*, 2011; Evans *et al.*, 2008; Gonzalez *et al.*, 2019).

The most common resistance mechanism of *Pseudomonas* pathogens is the antibiotic efflux pump mechanism. Although this mechanism is most often seen in *P. aeruginosa*, it is also found in other *Pseudomonas* pathogen genomes. It has long been known that the antibiotic efflux pump is a key mechanism of resistance

in Gram-negative bacterial pathogens (Blair *et al.*, 2014; Blanco *et al.*, 2016; Soto, 2013; Webber & Piddock, 2003). An antibiotic resistance strain's efflux pumps allow it to regulate itself by excluding toxic substances, including antimicrobial drugs (Blair *et al.*, 2014; Li *et al.*, 2015; Soto, 2013; Webber & Piddock, 2003).

Conclusion

The different ARGs and mechanisms against known drugs in *P. aeruginosa* in comparison to other *Pseudomonas* pathogen

were investigated and concisely reported in this brief report. The findings in this report could be useful in understanding the use of chemotherapeutics against antibiotic-resistant strains of *Pseudomonas* pathogens.

Data availability

Source data

Table 1 lists the NCBI Genome accession numbers used in this study.

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Paula Blanco

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This brief report deals with the comparison of five *Pseudomonas* species' genomes in order to get information about the different antibiotic resistance genes and mechanisms. However, the findings described here lack novelty and interest, since the mechanisms of resistance and antimicrobial resistance genes have been widely described in the different *Pseudomonas* species along the literature.

Another point to consider is the number of genomes used for the work. Five complete genomes as a representation of the *Pseudomonas* pathogens group are not enough for drawing conclusions. It would be much more interesting to take a larger sample of genomes (since they are available) including more than a strain per *Pseudomonas* species, as well as environmental and clinical strains.

A more extensive and careful use of literature should be used along the manuscript, since there are some sentences that does not include a reference. For instance "The most common resistance mechanism of *Pseudomonas* pathogens is the antibiotic efflux pump mechanism". Also, one of the references for the sentence "*P. aeruginosa* is of great medical importance due to its exhibition of multidrug resistance and its association with serious illnesses" mention a work performed in the nematode *C. elegans*. It would be right to include more references related to the clinics.

Other aspects of this brief report should need clarification, for instance, what is RG in Table 1? What is the difference between perfect and strict cutoff in Fig1A? Figure 2B also needs clarification, since it is difficult for the reader to make an interpretation and get any conclusion. The author claim in the conclusion that the "findings of this brief report could be useful in understanding the chemotherapeutics against antibiotic resistance strains of *Pseudomonas* pathogens". However, it is not discussed how actually these data could be used or applied. The author should also mention other similar in silico studies already published to compare his results and give consistency to the methodology employed here.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antimicrobial resistance evolution

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 19 August 2020

<https://doi.org/10.5256/f1000research.28015.r68814>

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Asad U. Khan

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Short communication focuses on antibiotic resistance caused by efflux pump overexpression and the use of cryo electron microscopy for protein protein or protein ligand interaction. It also describes the limitations of x ray crystallography. The author also extends the cryo EM use in interparticle motions of the ribosome in different tRNA bound states, its visualization in various conformations upon the introduction of ribosomal-specific substrates and several bacterial membrane proteins to near atomic resolution. Author successfully explain the utility of structural dynamics in drug designing by identifying important residues through solved structures. However, references are missing from the introduction and targeting efflux pumps to mitigate antibiotic resistance section. May be accepted after minor revision.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

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