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ORIGINAL ARTICLE

Comparative genomic analysis of *Pseudomonas aeruginosa* strains susceptible and resistant to carbapenems and aztreonam isolated from patients with healthcare-associated infections in a Mexican hospital

María José Martínez-Gallardo[1] · Claudia Villicaña[2] · Martha Yocupicio-Monroy[3] · Sofía Lizeth Alcaraz-Estrada[4] · Juana Salazar-Salinas[5] · Omar Fernando Mendoza-Vázquez[5] · Gabriel Damazo-Hernández[5] · Josefina León-Félix[1]

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Abstract

Pseudomonas aeruginosa (PA) is an important opportunistic pathogen that causes different infections on immunocompromised patients. Within PA accessory genome, differences in virulence, antibiotic resistance and biofilm formation have been described between strains, leading to the emergence of multidrug-resistant strains. The genome sequences of 17 strains isolated from patients with healthcare-associated infections in a Mexican hospital were genomically and phylogenetically analyzed and antibiotic resistance genes, virulence genes, and biofilm formation genes were detected. Fifteen of the 17 strains were resistant to at least two of the carbapenems meropenem, imipenem, and the monobactam aztreonam. The antibiotic resistance (*mexA*, *mexB*, and *oprM*) and the biofilm formation (*pslA* and *pslD*) genes were detected in all strains. Differences were found between strains in accessory genome size. The strains had different sequence types, and seven strains had sequence types associated with global high risk epidemic PA clones. All strains were represented in two groups among PA global strains. In the 17 strains, horizontally acquired resistance genes to aminoglycosides and beta-lactams were found, mainly, and between 230 and 240 genes that encode virulence factors. The strains under study were variable in terms of their accessory genome, antibiotic resistance, and virulence genes. With these characteristics, we provide information about the genomic diversity of clinically relevant PA strains.

Keywords *Pseudomonas aeruginosa* · Nosocomial infections · Multidrug resistant · Pangenome

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✉ Josefina León-Félix
ljosefina@ciad.mx

María José Martínez-Gallardo
maria.martinez17@estudiantes.ciad.mx

Claudia Villicaña
maria.villicana@ciad.mx

Martha Yocupicio-Monroy
martha.ym1@gmail.com

Sofía Lizeth Alcaraz-Estrada
sofializeth@gmail.com

Juana Salazar-Salinas
jua.salazar@issste.gob.mx

Omar Fernando Mendoza-Vázquez
qfb23omv@outlook.com

Gabriel Damazo-Hernández
gdz14@hotmail.com

[1] Laboratory of Molecular Biology and Functional Genomics, Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD), Culiacán, Sinaloa, Mexico

[2] CONACYT-Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD), Culiacán, Sinaloa, Mexico

[3] Postgraduate in Genomic Sciences, Universidad Autónoma de la Ciudad de México (UACM), Mexico City, Mexico

[4] Division of Genomic Medicine, Centro Médico Nacional 20 de Noviembre-ISSSTE, Mexico City, Mexico

[5] Central Epidemiological Surveillance Laboratory-ISSSTE, Mexico City, Mexico

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Introduction

Currently, most antibiotics have become ineffective for the control of bacterial infections, as bacteria have acquired multiresistance to these kinds of drugs due to the indiscriminate and widespread use of antibiotics (Shahi and Kumar 2015). These multidrug-resistant (MDR) pathogens have been called "superbugs" and have become one of the most critical public health problems causing more than 1 million deaths per year worldwide (CDC. Antibiotic Resistance Threats in the United States 2019; Antimicrobial Resistance Collaborators 2022). The term "ESKAPE" encompasses six of these pathogens with increasing multidrug resistance and virulence: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* (PA), and *Enterobacter* spp. ESKAPE pathogens are responsible for most nosocomial infections and are able to "escape" the biocidal action of antimicrobial agents (Mulani et al. 2019).

The World Health Organization (WHO) reports that 50% of infections caused by *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* show resistance to antibiotics such as cephalosporin, an improved beta-lactam antibiotic. This group of bacteria induces healthcare-associated infections (HAIs) or nosocomial infections, which are defined as infections acquired by a patient during treatment in a hospital that a patient does not have at the time of admission (PAHO 2012). HAIs affect 1 in 20 hospitalized patients, and in Mexico the yearly death toll attributed to HAI is 37,000 by this kind of infections (IMSS 2019).

In Mexico, the epidemiological surveillance of HAIs is under the charge of the Hospital Epidemiological Surveillance Network. Additionally, the Institute for Social Security and Services for State Workers (ISSSTE) carries out its control and surveillance through the Committee for the Detection and Control of Nosocomial Infections, finding PA among the first microorganisms causing HAIs (RHOVE 2021).

Pseudomonas aeruginosa is a Gram-negative bacterium established in water, soil, and various host organisms. In humans, it is considered the fourth most isolated nosocomial pathogen, accounting for 10% of all HAIs, and the second most common cause of pneumonia (IMSS 2019). PA is characterized by its ability to generate resistance to antibiotics, through gene mutation or by transferring horizontally acquired resistance genes (Ozer et al. 2014). Both clinical and environmental strains can thrive in a wide variety of ecological niches and cause damage in different hosts, as it is a versatile bacterium with easy adaptability due to the maintenance of virulence and metabolic genes in its genome. The importance of this bacterium lies in the fact that it causes significant morbidity and mortality among immunocompromised patients and those requiring mechanical ventilation, such as burn patients and those with cystic fibrosis (CF). Some multilocus sequence types (ST) referred to as high-risk clones are extensively issued throughout the world and frequently found in PA (Valot et al. 2015).

The increased availability of genomes has made it possible to perform comparative genomic analyses of PA strains in distinct parts of the world (Ozer et al. 2014; Jeukens et al. 2014; Subedi et al. 2018). These studies have defined the pangenome of strains with a "core genome", which is all the genes present, and an "accessory genome" which is the set of genes not shared among all members of the species (Ozer et al. 2014). The PA genome has a size of 5.5–7 Mbp; this variation is due to the presence of an accessory genome that is specific to each strain and can occupy up to 20% of the total genome. This accessory genome consists of horizontally transferable elements including prophages, plasmids, insertion sequences (IS), genomic islands (GI), and transposons. The accessory genome is significant for bearing acquired antibiotic resistance and virulence genes, with lateral transfer of these genes among strains contributing to the development of more virulent strains. Additionally, mutational changes may promote antibiotic resistance and virulence. Genome characterization studies of PA isolates such as those by Aggarwal et al. (2015) and Subedi et al. (2018) have shown that the pangenome contains large heterogeneity in accessory genome size among 22 strains which was correlated with the quantity of prophages, insertion sequences, and genomic islands. The strains from these studies had different sequence types and were distinct from the worldwide PA epidemic clones. The isolates previously characterized have genes that confer resistance to aminoglycosides, beta-lactams, quaternary ammonium compounds, sulfonamides, chloramphenicols and tetracyclines. We know that PA genome aids in comprehending the genetic modifications that are related to more resistant and pathogenic strains, however there is little information on comparative genomic analysis of PA strains isolated in Mexico. Hence, the objective of this study was to compare the genomic variability within PA strains isolated from hospitalized patients with different HAIs from the Centro Medico Nacional 20 de Noviembre in Mexico City, for which the complete genomes of 17 strains were sequenced and genomic analysis was performed by comparing evolutionary relationships, genomic divergence, antibiotic resistance, and virulence factors.

Materials and methods

Bacterial strains and antibiotic susceptibility testing

We studied 17 clinical strains of *Pseudomonas aeruginosa* provided by the Central Epidemiological Surveillance Laboratory, Centro Medico Nacional 20 de Noviembre ISSSTE of Mexico City, isolated only from this hospital from samples of different patients with HAIs between the years 2019 and 2021, as shown in Table 1. The disk diffusion method was performed based on the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI) to determine antibiotic susceptibility patterns, using PA ATCC 27853 as control strain. The antibiotic disks (BD BBL, USA) used in this study included meropenem (10 µg), imipenem (10 µg), and aztreonam (30 µg). The isolates with resistance to any of the two carbapenems (meropenem and imipenem) were considered as PA resistant to carbapenems, and the isolates with resistance to aztreonam were considered as PA resistant to monobactams (Magiorakos et al. 2012).

DNA extraction and PCR detection of resistance and biofilm formation genes

The strains were grown in 5 ml of TSB liquid medium at 37 °C for 24 h and genomic DNA was extracted following the method described by Chen and Kuo (1993). PCR reactions for detection of efflux pump (*mexA*, *mexB*, and *oprM*) and biofilm-forming (*pslA* and *pslD*) genes were done using specific primers listed in Table S1. Reactions were carried out in 0.2 mL microcentrifuge tubes in a reaction volume of 25 µL using Go Taq PCR CORE systems kit (Promega)

Table 1 *Pseudomonas aeruginosa* strains isolated from clinical samples of patients with different HAIs and their antibiotic susceptibility profile

Halo size (mm)	Antibiotics (carbapenem class)					Isolation year	Associated HAI	Type of sample	Code
	Interpretation Aztreonam	Halo size (mm)	Interpretation Imipinem	Halo size (mm)	Interpretation Meropenem				
27	S	22	S	31	S	1971	No data	Blood culture	ATCC 27853
6	R	6	R	6	R	2019	UTI	Urine	19,021
18.6	I	10.4	R	18.6	I	2019	RTI	Sputum	19,029
6	R	6	R	6	R	2019	STI	Blood culture	19,071
23.8	S	6	R	6	R	2019	STI	Blood	19,121
16.7	R	6	R	6	R	2019	RTI	Bronchial secretion	19,133
21.7	S	23.7	S	27.4	S	2019	No data	No data	19,182
14	R	11.5	R	10	R	2019	No data	No data	19,199
25	S	24	S	24.2	S	2019	SSI	Wound secretion	19,205
21.5	I	6	R	6	R	2019	STI	Blood	19,241
23.8	S	6	R	6	R	2019	SSI	Wound secretion	19,256
20.4	I	6	R	6	R	2019	Catheter site	Catheter site discharge	19,291
15.3	I	6	R	6	R	2019	SSI	Abdominal secretion	19,299
0	R	0	R	0	R	2020	RTI	Bronchial aspirate	20,019
20	I	14	R	18	R	2020	STI	Blood	20,061
0	R	0	R	0	R	2020	UTI	Urine	20,238
21	I	0	R	0	R	2021	RTI	Bronchial secretion	21,049
15	R	8	R	7	R	2021	IIA	Peritoneal dialysis fluid	21,058

UTI urinary tract infection, *RTI* respiratory tract infection, *STI* bloodstream infection, *SSI* surgical site infection, *IIA* intra-abdominal infection, *R* resistant, *I* intermediate, *S* susceptible

containing 5× buffer, 25 mM $MgCl_2$, 10 mM nucleotide triphosphates (dNTPs), 0.1 µM primers and1-unit Taq DNA polymerase (GoTaq Promega). PCR conditions consisted of one cycle at 95 °C for 5 min; 30 cycles of 30 s at 95 °C, 40 s at Tm specific for each primer, 1 min at 72 °C and a final elongation at 72 °C for 10 min. The PCR products were separated on a 1% ethidium bromide agarose gel with 1X TAE (70 v, 300 mA, 1 h) and visualized using UV light transilluminator (Ugwuanyi et al. 2021).

Whole genome sequencing

Once a 3-µg sample of genomic DNA with a purity of 1.8 (absorbance ratio of 260/280 nm) by the method described previously was obtained and its integrity verified by 1% agarose gel electrophoresis, the samples were sequenced by service at the Laboratorio Nacional de Genómica para la Biodiversidad del Centro de Investigación y de Estudios Avanzados del IPN (LANGEBIO, CINVESTAV-IPN, Irapuato Unit). Seventeen of 137 (12.4%) of the isolates collected as part of surveillance were sequenced. The selection criteria for the isolates to be sequenced were strains that had different susceptibility to the carbapenems and aztreonam tested and that they were strains isolated from different years. The sequencing of the genetic material was carried out by the sequencing-by-synthesis method using a Truseq DNA nano kit with the Illumina Miseq system in 2 × 150 bp format.

Genomes assembly and analysis

MiSeq sequencing resulted in a range of 653,270 to 2,199,951 reads per sample with an average coverage of 140×. With FastQC version 0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc), the quality of the raw reads was evaluated, and Trimmomatic version 0.36 (Bolger et al. 2014) was used to remove adapters with default parameters (SLIDINGWINDOW:4:30 MINLEN:36). A de novo genome assembly was done using SPAdes version 3.13.1 (Bankevich et al. 2012) using the default settings. Prokka version 1.14.5 was then used for annotations of the assembled genomes (Seemann 2014). The PA PAO1 genome (RefSeq accession number NC_002516.2) was used as a reference in the present work. The preliminary genome contigs were rearranged and aligned to the reference genome with the MAUVE multiple genome alignment software (Darling et al. 2010; Rissman et al. 2009). Genomic islands were determined using IslandViewer (Bertelli et al. 2017), and PHASTER to identify and annotate prophages (Arndt et al. 2016). The pubMLST database was used to determine the multilocus sequence type, selecting *Pseudomonas aeruginosa* as an organism, single sequence and uploading the fasta file of each genome (Jolley et al. 2018). The completed genome sequence of the strains has been deposited in the GenBank database under accession number PRJNA1040412 (http://www.ncbi.nlm.nih.gov/bioproject/1040412).

Pangenome and phylogenetics

Pangenome analysis was done with Roary version 3.13.0 (Page et al. 2015). Common genes in at least 99% of the strains were considered core genes and cloud, shell and soft core genes corresponding to gene families with presence in < 15%, 15–95%, 95–99%, respectively. The accessory genome was obtained by subtracting the core genes from the total genes of the genome of each strain. Two hundred fifty-two genomes of PA strains (235 previously reported complete genomes downloaded from The Pseudomonas Genome Database and 17 genomes from this work) were aligned with Parsnp version 1.2 having the PAO1 strain sequence as a reference, and then it was used to make a maximum likelihood tree in Mega X, taking in account the core genome single nucleotide polymorphisms (SNPs).

Screening of antibiotic resistance and virulence genes

The genomes of PA isolates were screened to identify acquired antibiotic resistance genes by using Resfinder 3.0 (Centre for Genomic Epidemiology, DTU, Denmark) (Zankari et al. 2012). Virulence genes associated with adherence, quorum sensing, type IV secretion system, alginate production, toxins and protease production were searched in the genomes using the virulence factor database (VFDB) uploading the fasta file of each genome and selecting the genus *Pseudomonas* (Chen et al. 2005).

Results

Antibiotic susceptibility profile

Seven of the 17 strains had antibiotic resistance to imipenem and meropenem, 8 strains had intermediate resistance to aztreonam, while 2 strains were susceptible to the three antibiotics tested. The strains showed greater resistance to imipenem and meropenem (carbapenems), compared to aztreonam (monobactam), irrespective of the year of collection (Table 1).

General characteristics of genomes and detection of antibiotic resistance and biofilm formation genes

The contigs generated by de novo assembly of the 17 PA strain genomes ranged from 96 in strain 19,256 to 754 in strain 20,019. The genomes in this study had a size of

6.6–7.3 Mbp and a mean C+G content of 65.95%. Genome size ranged extensively between strains, with up to 1.0 Mbp more DNA than the PAO1 strain. The number of coding sequences (CDS) oscillates from 5969 (strain 19,029) to 6844 (strain 19,133). The general characteristics of the genomes are shown in Table 2. Genotypic evaluation showed that all antibiotic resistance genes (*mex*A, *mex*B and *opr*M) and biofilm formation genes (*psl*A and *psl*D) were detected in the 17 strains as shown in Fig. S1 and these five PCR-amplified genes presented above (*mex*A, *mex*B, *opr*M, *psl*A and *psl*D) were manually searched and found to be present in all the genomes of the strains.

A total of 12,273 orthologs were obtained from analyzed strains in this study. Additionally, of the 12,273 genes shared by all strains, this study described 4813 genes that formed the core genome which was common in at least 99% of the strains, no soft-core genes were found (common in 95–99% of strains), and there were 2938 shell genes (common in15-95% of strains) and 4521 cloud genes (common in 0–15% of strains) (Fig. 1).

The accessory genome ranged from 19 to 29% of the complete genome. In these accessory genomes, genes were found associated with antibiotic resistance and virulence (beta-lactamases, multidrug efflux pumps, toxins, transposases, integrases), as well as genes that aid strains to survive in hostile environments like strains cultured from sites containing toxic organic compounds and heavy metals which are unsuited for PA habitation (hypothetical proteins mainly).

As elements of accessory genome, the results indicate that the predicted number of genomic islands (GI) ranged from 27 to 90 and that of of prophages from 1 to 5 in the 17 genomes (Table S2).

According to the multilocus sequence typing (MLST) analysis, ten different sequence types (ST) were found in the 17 genomes, and only one constituted a new type. The results indicate that five isolates belonged to ST 233, three isolates to ST 309 and two strains to ST 3045 (sequence types determined by MLST database are listed in Table 2), while the remaining strains corresponded to seven different STs.

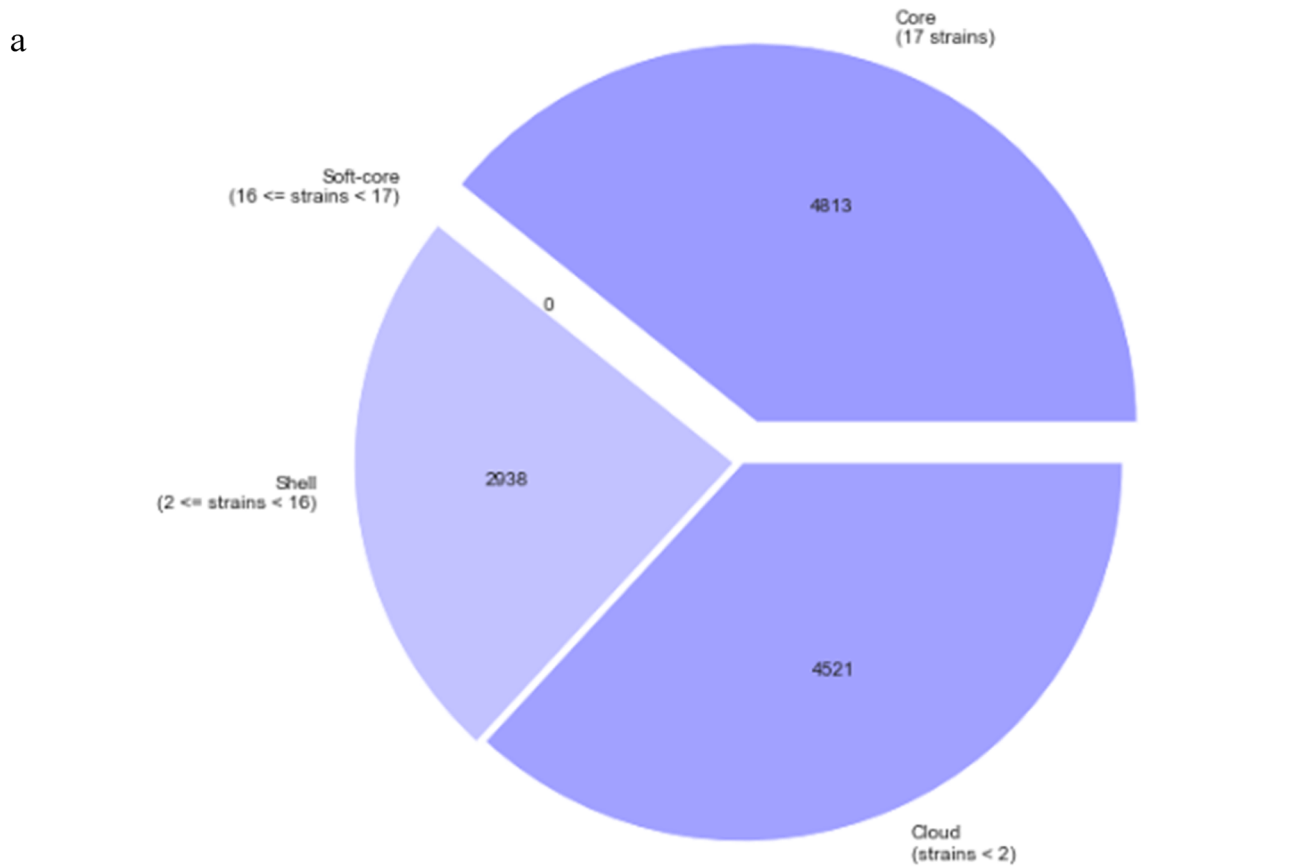
Phylogenetics

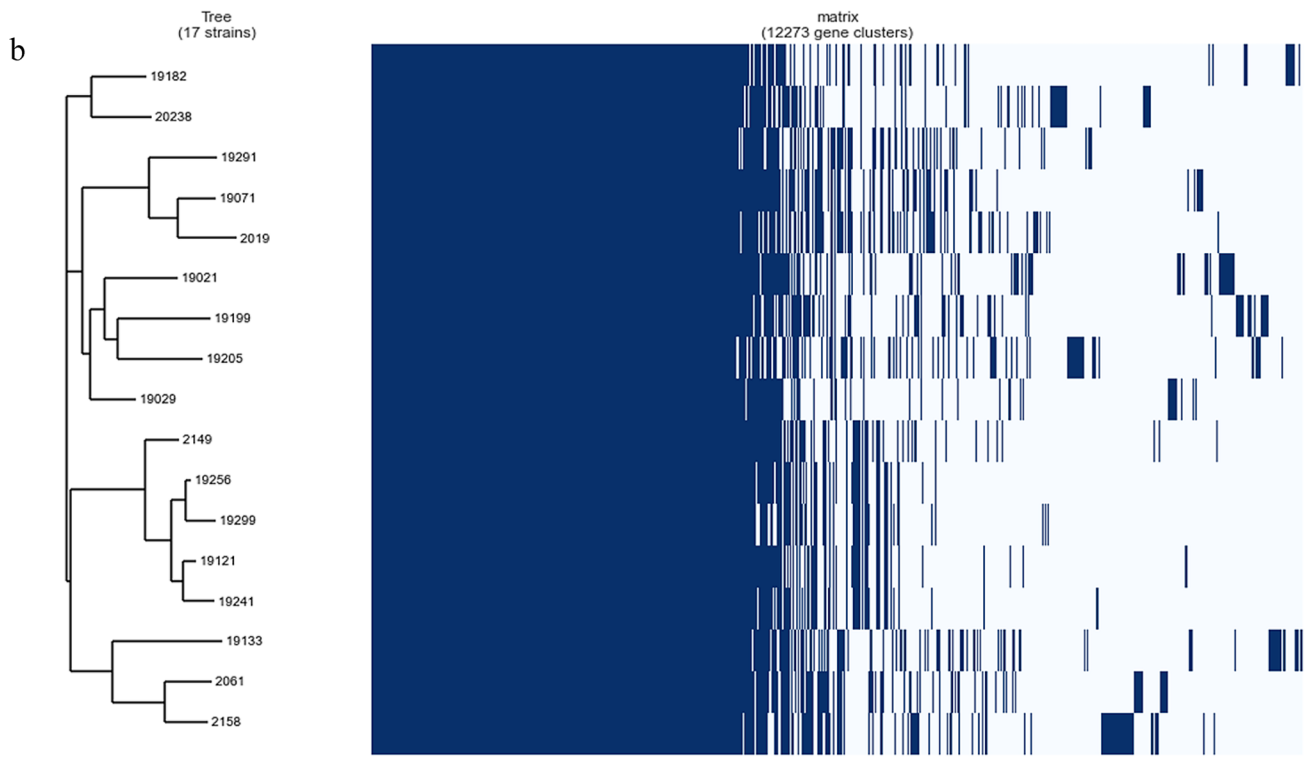
The strains were not clonally related and were well represented in two groups, nine strains in group 1 and eight in group 2 (Fig. 2). Interestingly, three groups of strains that were grouped by clade had the same ST in each clade. Strains 19,121, 19,241, 19,256, 19,299 and 21,049 were grouped within group 1 and had ST 233 reported as high-risk clones, also within the group. Group 1 strains 20,061 and 21,068 had ST 3045 and, within group 2, strains 20,019, 19,071 and 19,291 with ST 309 were grouped in the same clade. This could indicate potential transmission, but it would be necessary to analyze both phylogenetic distance and epidemiological link.

Table 2 General characteristics of the *Pseudomonas aeruginosa* strains genomes

Strain	Sequence type*	No. of contigs	Length (bp)	%GC	CDS	tRNA	Accesory genes	% Accesory genome
19,021	New	224	7,020,763	65.81	6474	65	1661	24.1
19,029	235	217	6,600,218	66.31	5969	69	1156	17.7
19,071	309	546	7,073,259	65.88	6496	64	1683	24.4
19,121	233	101	6,933,644	65.99	6302	68	1489	22
19,133	111	486	7,324,582	65.68	6844	69	2031	28.2
19,182	27	218	6,651,343	66.13	6104	68	1291	19.6
19,199	308	317	7,162,628	65.79	6617	67	1804	25.7
19,205	532	659	7,003,379	65.78	6427	73	1614	23.6
19,241	233	552	6,893,744	65.93	6297	67	1484	22
19,256	233	96	6,898,377	65.99	6281	68	1468	21.8
19,291	309	222	7,018,393	65.91	6418	67	1605	23.4
19,299	233	727	6,840,867	65.9	6240	65	1427	21.3
20,019	309	754	7,094,083	65.87	6539	71	1726	24.9
20,061	3045	532	7,153,863	65.69	6607	70	1794	25.6
20,238	17	301	7,002,407	65.85	6439	72	1626	23.7
21,049	233	251	6,966,072	65.98	6360	68	1547	22.7
21,058	3045	686	7,349,074	65.3	6800	70	1987	27.7

*Sequence types were determined by the multilocus sequence typing database. Sequence types not listed in the MLST database have been considered as new


a
Core
(17 strains)
4813
Soft-core
(16 <= strains < 17)
0
Shell
(2 <= strains < 16)
2938
4521
Cloud
(strains < 2)


b
Tree
(17 strains)
19182
20238
19291
19071
2019
19021
19199
19205
19029
2149
19256
19299
19121
19241
19133
2061
2158
matrix
(12273 gene clusters)

◂**Fig. 1** Pangenome of the 17 PA strains studied. **a** Pie chart of the breakdown of genes. The pangenome is subdivided into core and accessory genes. The accessory genome is further subdivided into cloud and shell genomes, which are based on the number of genomes that contain the gene(s). The number of genes present in each category are shown. The number of strains necessary for a gene to meet the defined category is shown in parentheses. **b** A tree compared to a matrix that represents the gene content, in terms of presence/absence of core and accessory genes of the pangenome inferred from the comparison of the 17 PA complete genomes

Genetic profiles of antibiotic resistance and virulence genes

A total of 45 different acquired antibiotic resistance genes were found in the present work using ResFinder database (Fig. 3). Most resistance genes associated with aminoglycosides and beta-lactams were observed in the strains from this study. The genes *blaPAO* (resistance to beta-lactams), *fosA* (resistance to fosfomycin), *aph(3')-IIb* (resistance to aminoglycosides) and *catB7* (resistance to chloramphenicol) were detected in the 17 strains. The *aph(3')-IIb* gene was the most common aminoglycoside, being found in all strains except 19,256 strain. One strain (19,291) had five resistance genes that were not detected in other strains; *aph(6)-Id*, *aph(3')*-VIa, *aph(3'')-Ib* aminoglycoside, $bla_{\text{IMP-15}}$ and *cat*A1 that confer resistance to aminoglycosides, beta-lactams and phenicols.

Virulence genes related with adherence, quorum sensing, type IV secretion system, alginate production, toxins and protease production were searched and found between 230 and 240 genes that encode virulence factors in our 17 PA genomes using the Virulence Factor Database (VFDB). Differences in virulence genes were found for genes involved in adherence, iron uptake and secretion systems (Table 3).

Discussion

A total of 17 strains of PA isolated from different samples of hospitalized patients with HAIs were obtained. Although most of the strains were resistant to more than one antibiotic, it would be appropriate to use a greater number of strains from 2020 and 2021 to make a fair comparison since most of the strains were isolated from the year 2019. Also, carbapenems are described as the most active drugs against PA (Bajpai et al. 2019), so it is important to highlight that most of the strains in this study were resistant to this class of antibiotics, which points to the need to find new alternatives to control this pathogen. Jeukens et al. (2017) reported that seven strains from CF patients were mostly susceptible to imipenem and meropenem, but resistant to ampicillin and amoxicillin; however, these strains were isolated in 1993, so it can be inferred that the bacterium have become more resistant over the years. The emergence of *Pseudomonas* species resistant to carbapenems and monobactams has been reported in several studies. Bajpai et al. (2019) found 126 isolates resistant to multiple kinds of antibiotics, with greater resistance to meropenem, 92/126 (73%); aztreonam, 87/126 (69%); and imipenem, 78/126 (61.9%). In another work, Subedi et al. (2018) reported 22 susceptible and intermediate resistance strains to imipenem and aztreonam (carbapenem and monobactam, respectively), but resistant to gentamicin (aminoglycoside) and ciprofloxacin (quinolone); so, susceptibility to antibiotics may vary according to the classes of antibiotics.

Given the high levels of resistance present in the strains under study, the detection of some genes involved in antibiotic resistance and biofilm formation was sought. For example, the *mex*AB-*opr*M efflux pump system has been reported to be very common and aids in PA intrinsic resistance to β-lactams (including carbapenems); thus, the genes (*mex*A, *mex*B and *opr*M) related to this antibiotic resistance system were searched for in the 17 strains. Also were searched the biofilm formation genes (*psl*A and *psl*D) that have been reported to enhance biofilms formation that promote resistance to antibiotics (Ugwuanyi et al. 2021). Our results coincide with the previous work of Ugwuanyi et al. (2021) where they describe that the *mex*A, *psl*A and *psl*D genes were detected in 39 (100%) isolates, but not the *mex*B and *opr*M genes, which were identified in 34–36 isolates (88–92%), respectively. However, Abbas et al. (2018) reported the detection of *mex*AB-R in 100% of PA isolates from urinary tract infections in Egypt.

In the genomic characterization, the number of contigs and genome size were similar to those in other published PA genomes (Jeukens et al. 2014, 2017; Subedi et al. 2018; Hao et al. 2021). The genomes of the 17 strains isolated in this work contain more ortholog genes than those in the study of Subedi et al. (2018), which isolated 22 PA strains from various origins and tfound 9786 orthologs in the pangenome. The larger pangenome found in the present study may be due to the diverse type of samples of patients that were used to isolate the strains, since the size of the pangenome can increase depending on the diversity of the strains in the study and depicts the accumulated genetic information between a group of bacterial genomes.

Previous reports have informed core genomes of 5316 (6406 pangenes) (Ozer et al. 2014), 5233 (9344 pangenes) (Valot et al. 2015), and 4910 (9786 pangenes) (Subedi et al. 2018) in different strains of PA. Even though these studies used less (less than 17) genomes, the results can be compared. Finding the smallest core genomes in this study may be due to the use of more genomes for the alignment, as well as the use of incomplete draft genomes and definition of the core genome (≥ 99% similarity in each strain) (Subedi et al. 2018). Within the core genome were genes related to

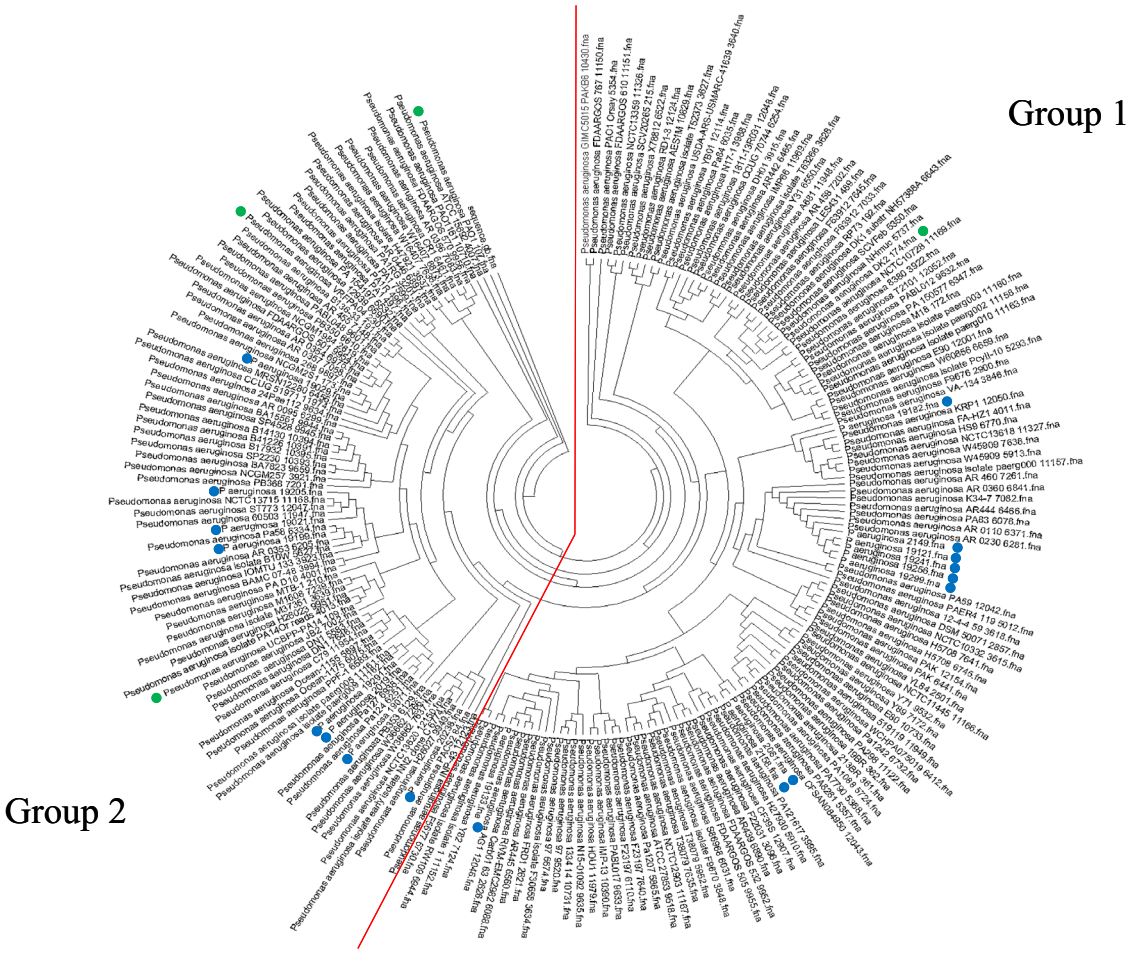


Fig. 2 Phylogenetic analysis of *Pseudomonas aeruginosa* isolates. Strains used in this study are indicated by a blue circle. The positions of some reference strains, such as *P. aeruginosa* VRFPA04, *P. aeruginosa* UCBPP-PA14, *P. aeruginosa* PAO1 and *P. aeruginosa* DK2, are shown by green circles

respiratory and metabolic genes that give the bacterium the ability to survive on a variety of carbon sources to obtain energy (Valot et al. 2015).

Besides the core genome, PA has an accessory genome that varies across strains, where most of these genes are acquired horizontally. In this work, accessory genes were calculated by removing the core genes (4813) from the number of CDS. The accessory genome of the strains in this study, which is larger than previously reported may be attributed to the use of draft genomes that may overestimate the number of accessory genes giving a seemingly larger genome (Subedi et al. 2018). Therefore, the increase in the number of accessory genes found in the present work may be because the strains have acquired many genes to be able to grow and survive in the hospital environment where, thanks to the contribution of hospital equipment and personnel, there is greater dissemination of resistance genes (Subedi et al. 2018). According to previously reported accessory genomes, a large number of undetermined genes were also found in this study, either because their function is unknown, or they are pseudogenes. It is important to highlight that in these accessory genomes, there were many genes that participate in replication, recombination, and repair, mainly integrases, recombinases and transposases, whose function is DNA mobility (Valot et al. 2015). As important elements of accessory genome, genomic islands (GI) and prophages were also identified and found in the genomes (Kung et al. 2010).

The ST was allocated to every strain; therefore if a strain did not coincide with the MLST database, it was taken as a new ST and is stored in the database after verification. These results indicate that the strains in the study belong to a wide variety of STs and coincide with previously clinical epidemic isolates reported worldwide (Aguilar-Rodea et al. 2017; Miyoshi-Akiyama et al. 2017; Witney et al. 2014; Woodford et al. 2011). The ST 233 was the most common ST found in this work, which has been reported in MDR hospital strains in different countries (Kocsis et al. 2021). Most

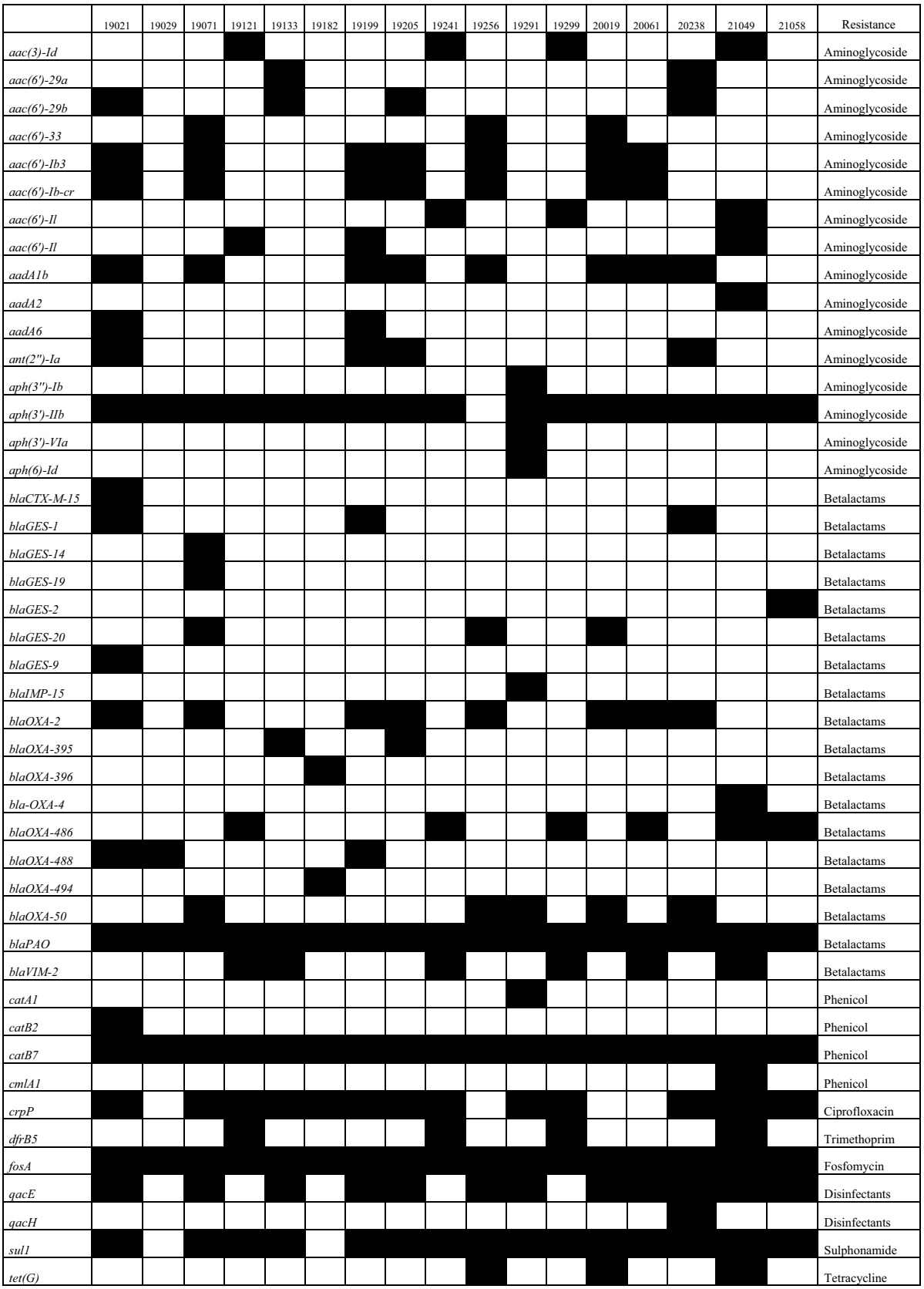


Fig. 3 Antibiotic resistance gene profile detected by Resfinder database. Associated resistance: aminoglycosides, betalactams, phenicols, ciprofloxacin, trimethoprim, fosfomycin, disinfectants, sulfonamide and tetracycline

Table 3 Virulence factors detected in the 17 PA strains analyzed in the VFDB

	19,021	19,029	19,071	19,121	19,133	19,182	19,199	19,205	19,241	19,256	19,291	19,299	20,019	20,061	20,238	21,049	21,058
Adherence	85	85	87	92	96	88	85	83	92	92	85	92	86	87	87	92	90
Antimicrobial activity	10	12	10	12	10	10	10	8	12	12	10	11	10	10	8	9	9
Antiphagocytosis	25	25	25	25	24	25	25	25	23	25	25	23	24	24	25	25	23
Biosurfactant	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Enzyme	4	4	4	3	3	3	4	4	3	3	4	3	4	4	3	3	4
Iron absorption	28	28	25	30	24	27	27	28	28	30	27	27	25	27	27	29	26
Protease	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Quorum sensing	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Regulation	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3
Secretion systems	65	63	60	64	63	62	60	61	60	64	62	60	60	62	62	64	59
Toxins	4	4	4	4	4	4	4	1	4	4	1	4	4	4	4	4	4
Total genes	235	235	229	244	240	233	229	224	236	244	229	234	227	232	230	240	232

frequently reported international high-risk MDR PA clones are ST 111, 175, 233, 235, 277, 357, 654, and 773 (Woodford et al. 2011; Kos et al. 2015; Kocsis et al. 2019). ST 233 has been described as a high-risk clone in Mexico, the USA, South Africa, and Japan. An ST 111 strain reported in Bulgarian hospitals was resistant to carbapenems and aztreonam (Kocsis et al. 2021), which coincides with the 19,133 strain that had the same ST and presented resistance to the three antibiotics.

The phylogenetic analysis coincides with previous studies where it has been described that PA strains from different sources fall into two main groups, where group 1 is the largest and includes the most investigate worldwide strains DK2, PAO1, LESB58 and PAK, while group 2 is slightly smaller and contains the studied virulent strain PA14 (Stewart et al. 2014).

In the identification of acquired antibiotic resistance genes, as in the 22 PA strains reported in the work by Subedi et al. (2018), the genes bla_{PAO} (resistance to b-lactams), *fosA* (resistance to fosfomycin), *aph(3')-IIb* (resistance to aminoglycosides) and *catB7* (resistance to chloramphenicol) were also detected in the 17 strains (100%) analyzed.

Differences in virulence genes were more evident for genes with the type III secretion system (*exoS*, *exoU*, *exoY* and *exoT*). The ExoT and ExoS toxins were the first to be identified in PA, participating in related functions such as altering the host cell actin cytoskeleton, inducing host cell rounding, inhibiting phagocytosis, and even causing cell death (Derakhshan et al. 2021). ExoY cleaves the intracellular cAMP in eukaryotic cells and causes cell rounding (Derakhshan et al. 2021). ExoU is considered the most virulent effector, as it disrupts eukaryotic membranes in different cell types. Based on these characteristics, PA strains can be classified into two groups: strains expressing ExoT and exoU cause rapid host cell death, while strains expressing *exoS* and *exoT* cause slow cell death. With the presence of *exoS* in ten strains in this study (59%), the strains can evade the action of antibiotics due to their ability to invade mammalian cells, while the presence of *exoU* in seven strains (41%) gives them a survival advantage by increasing resistance (Derakhshan et al. 2021). The *exoS* and *exoU* genes are mutually exclusive which was verified in the strains under study, since one of the two genes was found in each strain (Berthelot et al. 2003). The *exoU* gene is part of genomic islands, so the studied strains that possess *exoU* showed larger accessory genomes. The *exoY* and *exoT* genes have been described as the most prevalent secretory toxins in strains, being found in all (100%) and 15 (88%) of the strains, respectively (Fig. 4) (Subedi et al. 2018).

Regarding the type VI secretion system in PA, it has been reported that the phospholipase D gene (*pldA*) promotes chronic infections (Subedi et al. 2018); however, *pldA* was absent in 8 (47%) of the 17 isolates in this study (Fig. 4).

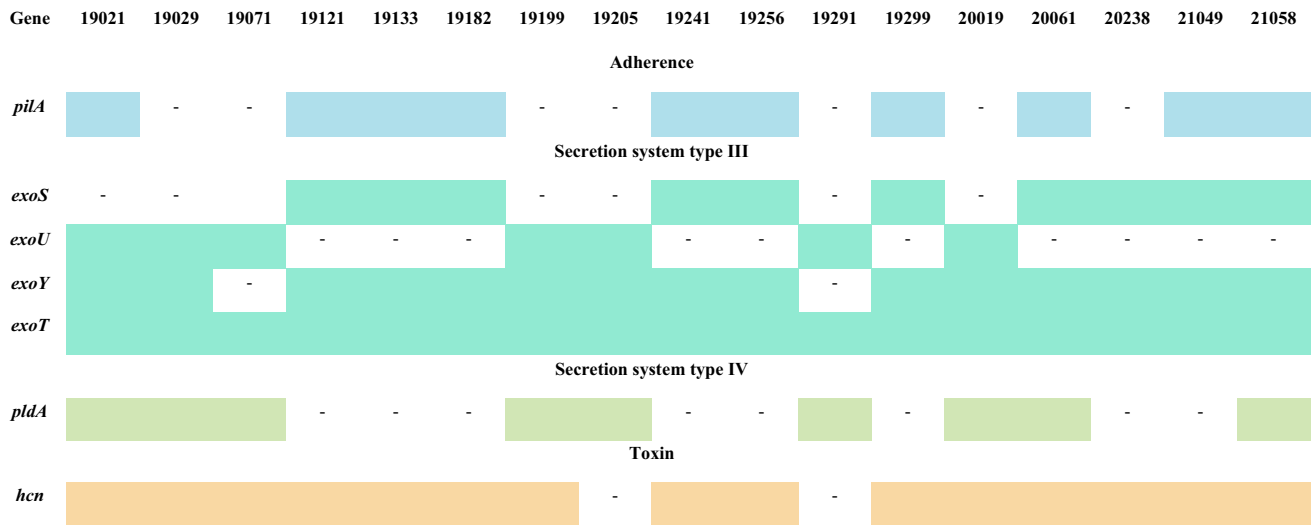


Fig. 4 Heterogeneity in virulence genes in PA genomes found for adherence genes (*pilA*), iron uptake (*hcn*), secretion system III (*exoS*, *exoU*, *exoY* and *exoT*), and secretion system IV (*pldA*)

Adhesion-associated genes such as type IV pili (*pilA*) are involved in adherence to and invasion of mucosal surfaces; consequently, *pilA* mutants exhibit less adherence and invasion of airway epithelial cells (Okuda et al. 2013). In this study, *pilA* gene was found in 10 (59%) of the 17 strains (Fig. 4), including some associated with respiratory tract infections.

Conclusion

In this study, a comparative genomic analysis was performed between PA isolates from patients with HAIs. The strains under study were different in terms of their accessory genome, antibiotic resistance, and virulence genes, and phylogenetically, the 17 strains were distributed throughout the two PA groups described. However, all strains have all the characteristics to become resistant to antibiotics used in hospitals, as well as possessing the necessary pathogenicity to cause infections in humans. Knowledge of the genes shared by most PA isolates makes it possible to search for, propose, and approve new therapies to combat the great diversity of human infections caused by this bacterium. Therefore, this information can be used to elucidate mechanisms or search for alternatives that help combat this virulent and multiresistant bacterium in the hospital environment.

Conflict of interest

The authors declare no competing interests.

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Author contributions All authors contributed to the study conception and design. Juana Salazar-Salinas, Omar Fernando Mendoza-Vázquez, and Gabriel Damazo-Hernández provided clinical isolates. Josefina León-Félix and María José Martínez-Gallardo designed the experiments. Experiment execution was performed by María José Martínez-Gallardo. Data analysis was performed by Josefina León-Félix, María José Martínez-Gallardo, and Claudia-Villicaña. The first draft of the manuscript was written by María José Martínez-Gallardo. Martha Yocupicio-Monroy and Sofía Lizeth Alcaraz-Estrada critically revised the work. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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