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Whole genome analysis of Gram-negative bacteria using the EPISEQ CS application and other bioinformatic platforms

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ABSTRACT

Objectives: To determine genomic characteristics and molecular epidemiology of carbapenem non-susceptible *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* from medical centres of Mexico using whole genome sequencing data analysed with the EPISEQ® CS application and other bioinformatic platforms.
Methods: Clinical isolates collected from 28 centres in Mexico included carbapenem-non-susceptible *K. pneumoniae* (n = 22), *E. coli* (n = 24), *A. baumannii* (n = 16), and *P. aeruginosa* (n = 13). Isolates were subjected to whole genome sequencing using the Illumina (MiSeq) platform. FASTQ files were uploaded to the EPISEQ® CS application for analysis. Additionally, the tools Kleborate v2.0.4 and Pathogenwatch were used as comparators for *Klebsiella* genomes, and the bacterial whole genome sequence typing database was used for *E. coli* and *A. baumannii.*
Results: For *K. pneumoniae,* both bioinformatic approaches detected multiple genes encoding aminoglycoside, quinolone, and phenicol resistance, and the presence of $bla_{\text{NDM-1}}$ explained carbapenem non-susceptibility in 18 strains and $bla_{\text{KPC-3}}$ in four strains. Regarding *E. coli*, both EPISEQ® CS and bacterial whole genome sequence typing database analyses detected multiple virulence and resistance genes: 20 of 24 (83.3%) strains carried bla_{NDM}, 3 of 24 (12.4%) carried $bla_{\text{OXA-232}}$, and 1 carried $bla_{OXA\text{-}181}$. Genes that confer resistance to aminoglycosides, tetracyclines, sulfonamides, phenicols, trimethoprim, and macrolides were also detected by both platforms. Regarding *A. baumannii,* the most frequent carbapenemase-encoding gene detected by both platforms was $bla_{\text{OXA-72}}$, followed by $bla_{\text{OXA-66}}$. Both approaches detected similar genes for aminoglycosides, carbapenems, tetracyclines, phenicols, and sulfonamides. Regarding *P. aeruginosa,* bla_{VIM}, bla_{IMP}, and bla_{GES} were the more frequently detected. Multiple virulence genes were detected in all strains.
Conclusion: Compared to the other available platforms, EPISEQ® CS enabled a comprehensive resistance and virulence analysis, providing a reliable method for bacterial strain typing and characterization of the virulome and resistome.

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1. Introduction

Drug-resistant Gram-negative bacteria—including *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii*, and *Pseudomonas aeruginosa*—are among the more frequent causative agents of healthcare-associated infections [1]. Several reports have described an increase in carbapenem resistance in these species after the COVID-19 pandemic [2–4].

Carbapenems are frequently used to treat infections due to multidrug-resistant Gram-negative bacilli, including these four bacterial species [5,6]. Carbapenem resistance is caused primarily by the production of carbapenemase enzymes. According to the Ambler β-lactamase classification, they are classified into class A (serine β-lactamases), class B (metallo-β-lactamases), and class D (oxacillinases) [7].

The most frequently reported carbapenemase enzymes worldwide are variants of *K. pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), and carbapenem-hydrolyzing oxacillinase (OXA) [8,9]. The spread of antibiotic resistance encoded by these genes has been attributed to the lateral transfer of genetic material by mobile genetic elements, including plasmids [10–12].

The advances in whole genome sequencing (WGS) technologies have facilitated the analysis of the complete DNA sequence of diverse organisms, including bacteria. This analysis allows a broad vision of virulence and resistance to antimicrobials, including some antibiotics not commonly used [13]. Certainly, WGS is becoming the method of choice for outbreak analysis of microbial pathogens [13]. The main challenge with WGS for microbial strain evaluation is the conversion of raw sequencing data to processable results for surveillance analysis. With the widespread use of genomic approaches, there is a growing need for tools that streamline WGS analyses without the need for an expert in bioinformatics in clinical microbiology laboratories and the possibility to use these results for surveillance analysis.

Therefore, this study aimed to determine genomic characteristics and molecular epidemiology of carbapenem non-susceptible *K. pneumoniae, E. coli, A. baumannii*, and *P. aeruginosa* from medical centers of Mexico using WGS data analysed with the software application EPISEQ® CS and other bioinformatic platforms.

2. Materials and methods

2.1. Study design, *data collection, and analysis*

In this study, clinical isolates from 28 centers (26 hospital-based laboratories and 2 external laboratories) in Mexico were collected. All participating centers belong to the network Red Temática de Investigación y Vigilancia de la Farmacorresistencia.

Clinical isolates included carbapenem-resistant *K. pneumoniae, E. coli, A. baumannii*, and *P. aeruginosa.* Species identification was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing was performed using VITEK® 2 (bioMérieux, Marcy l'Etoile, France) and interpreted based on Clinical and Laboratory Standards Institute (CLSI) criteria [14]. Isolates that exhibited resistance or intermediate susceptibility to any of the carbapenems evaluated were selected for further analysis.

A total of 75 clinical isolates were selected for WGS: 26 were collected in the pre-COVID-19 pandemic period (January to March 2020), and 49 were collected during the pandemic (January to August 2021). Species included were *K. pneumoniae* (n = 22; 1 pre-pandemic and 21 pandemic), *E. coli* (n = 24; 10 pre-pandemic and 14 pandemic), *A. baumannii* (n = 16; 9 pre-pandemic and 7 pandemic), and *P. aeruginosa*, (n = 13; 6 pre-pandemic and 7 pandemic).

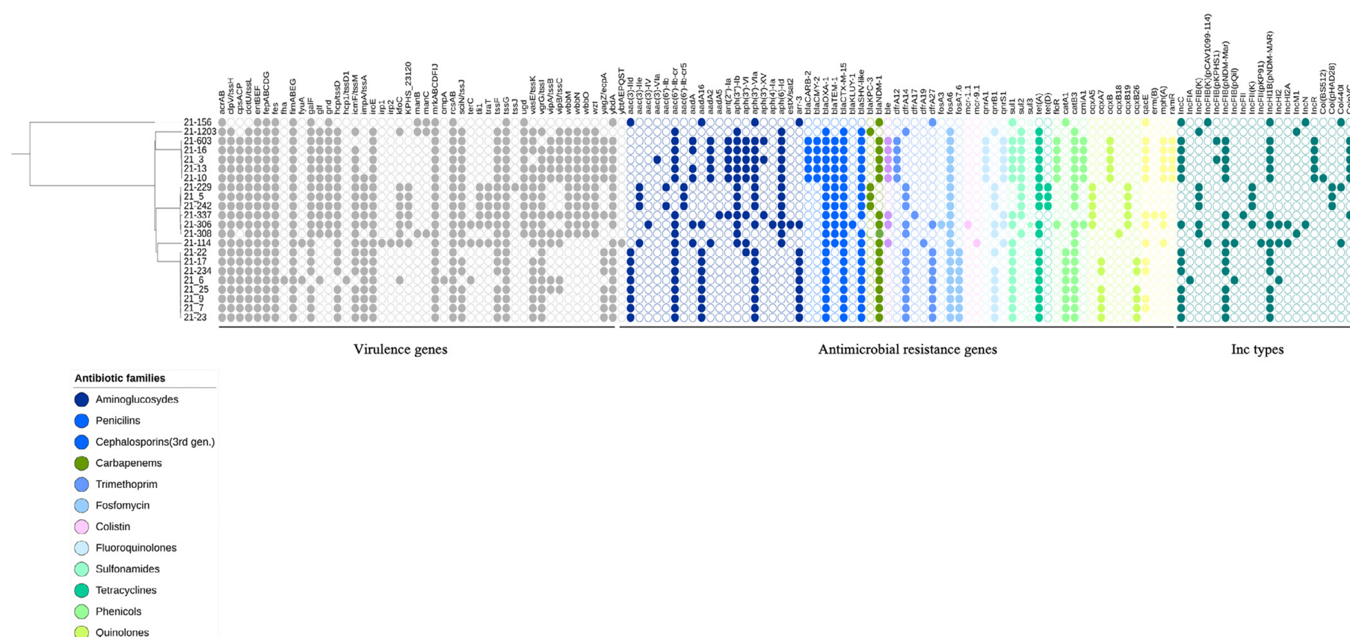

Fig. 1. Distribution of virulence and drug resistance genes and plasmid types in *K. pneumoniae* using the EPISEQ CS application.

2.2. Whole genome sequencing

All carbapenem non-susceptible isolates ($n = 75$) were subjected to WGS. DNA was extracted using the DNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. After extraction, the DNA concentrations were measured with a Qubit 3.0 (Thermo Scientific, Waltham, MA). From each DNA sample (average of 10 ng), 20 μL was transferred into a 96-well WGS plate. Sequencing libraries were generated using Nextera XT Illumina kits (San Diego, CA), and short-read sequencing was run on an Illumina (MiSeq) platform with a paired-end sequencing protocol (150-bp insert size) at the Laboratory of Cytogenomics and Microarrays of the Biochemistry Department, School of Medicine, Universidad Autónoma de Nuevo León.

The draft genome sequence was deposited in the National Center for Biotechnology Information (NCBI) GenBank database under project number PRJNA922195 .

2.3. EPISEQ® CS

After sequencing runs passed quality control checks, FASTQ files from the Illumina platform were uploaded to the easy-to-use, fully integrated web-based software application EPISEQ® CS, version 1.2.0 (bioMérieux). The application is based on a reference-free approach with automated workflow (https://www.biomerieux-episeq.com/cs-how-it-works). In brief terms, it checks the quality of sequencing data and uses the open-source algorithm SPAdes for genome assembly. Species identity, initially selected by the user, and potential intra- and inter-species contamination are checked. Genomic strain characterization was performed through the generation of multilocus sequence typing (MLST) results, serotyping and pathotyping for *E.coli* samples, and detection of antimicrobial resistance genes or point mutations (resistome) of virulence factors (virulome) and plasmids. Allele calling was performed in a proprietary whole-genome MLST (wgMLST) scheme defined for each bacterial species. A wgMLST-based epidemiological analysis was finally performed to compare clinical isolates. The number of loci for *K. pneumoniae* was 19 729 (including 1515 core loci), for *E. coli* was 17 380 (including 1291 core loci), for *A. baumannii* was 5633 (including 1393 core loci), and for *P. aeruginosa* was 15 143 (including 1480 core loci).

2.4. Genome Assembly and Data Analysis

De novo assembly was completed with SPAdes Genome Assembler v3.12.0 (Basespace Illumina). The draft genomes were annotated using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline. Kleborate v2.0.4 [15] was used to identify species, determine virulence loci, and pinpoint antimicrobial resistance genes (CARD database v3.0.8) [16] from assembled genomes of *K. pneumoniae*. Pathogenwatch was also used for *K. pneumoniae* genomes [17]. Analyses for *E. coli* and *A. baumannii* were also performed through the bacterial whole genome sequence typing database (BacWGSTdb 2.0) (http://bacdb.org/BacWGSTdb) [18]. Phylogenetic analysis of clinical isolate panels from each bacterial species was performed using BacWGSTdb, which gives a tree based on single nucleotide polymorphism strategy using default parameters. The calculated trees were exported in newick format and then visualized and edited with iTOL v6.5.2 (https://itol.embl.de/) [19].

3. Results

3.1. Antibiotic resistance

Regarding *K. pneumoniae*, all isolates had a minimal inhibitory concentration (MIC) $\geq$ 32 for ampicillin-sulbactam, $\geq$ 64 for ceftazidime and ceftriaxone, and $\geq$ 8 for doripenem. The MIC range for cefepime was 2–$\geq$ 64, 4– $\geq$ 8 for ertapenem, and 8– $\geq$ 16 for imipenem and meropenem. All susceptibility results are presented in Supplemental Table 1. For *E. coli,* all strains had an MIC $\geq$ 32 for ampicillin/sulbactam and $\geq$ 128 for piperacillin/tazobactam. The MIC range for ertapenem was 4– $\geq$ 8, 1– $\geq$ 16 for imipenem, and 1– $\geq$ 16 for meropenem. For *A. baumannii,* all strains had a MIC $\geq$ 128 for piperacillin/tazobactam, $\geq$ 64 for ceftriaxone, $\geq$ 8 for doripenem, $\geq$ 16 for imipenem and meropenem, and $\geq$ 4 for ciprofloxacin. For *P. aeruginosa*, all clinical isolates had a MIC $\geq$ 16 for imipenem. The MICs were 8– $\geq$ 128 for piperacillin/tazobactam,

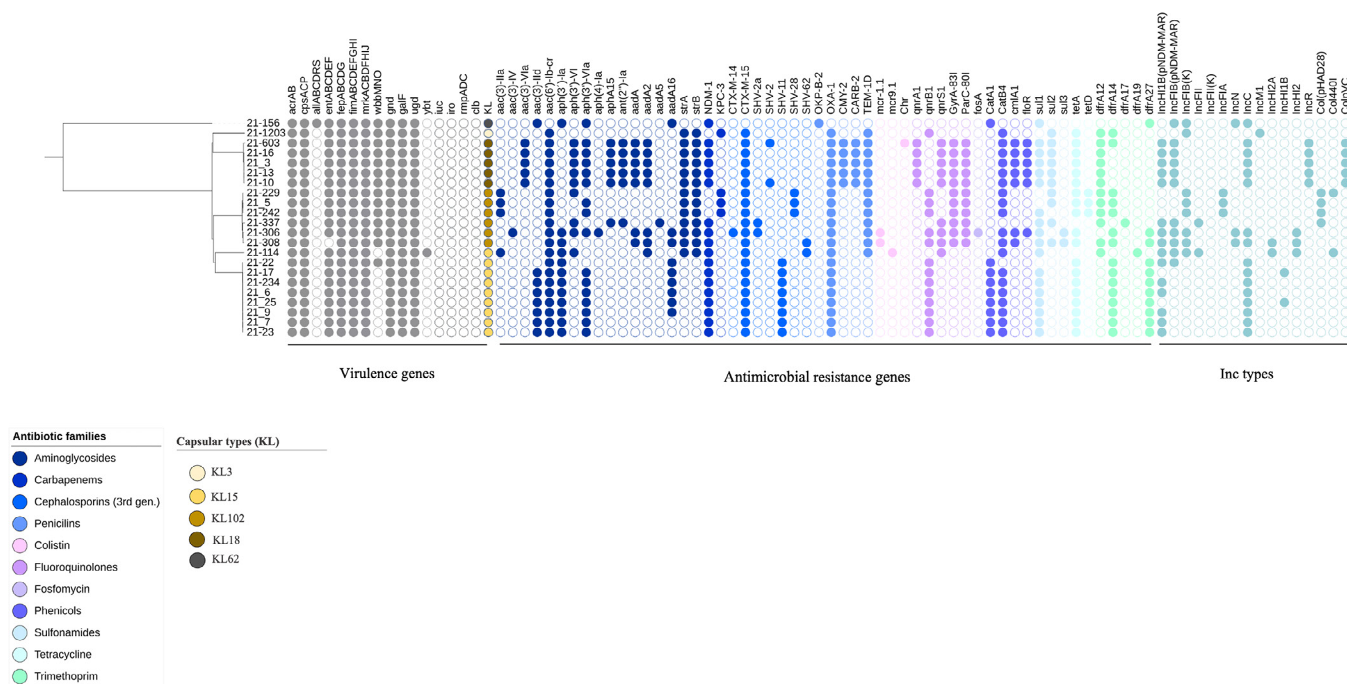

Fig. 2. Distribution of virulence and drug resistance genes and plasmid types in *K. pneumoniae* using the PathogenWatch and Kleborate patforms.

4 – ≥ 64 for cefepime, ≤ 0.12– ≥ 64 for doripenem, and 4– ≥ 16, for meropenem (Supplemental Table 1).3.2.

3.2. K. pneumoniae

For *K. pneumoniae* (n = 22), the genome size ranged from 5.54–6.62 million base pairs (Mbp; Supplemental Table 2). Regarding the virulome, the EPISEQ® CS application identified many virulence genes—such as *traT, terC, vipA*, and others—which were not detected using the Kleborate platform (Fig. 1 and Fig. 2). In contrast, the hypervirulence genes (*rmpADC, clb, iuc, iro*) were reported using the Kleborate platform; the capsular types were reported by Pathogenwatch platform reports; and these genes were not detected by the EPISEQ® CS application. The most frequent capsular type detected was KL15 (n = 9), followed by KL102 (n = 6), KL18 (n = 5), and KL3 and KL62 (n = 1 for each).

Regarding the resistome, both bioinformatic approaches detected multiple genes encoding aminoglycoside, quinolone, and phenicol resistance (Fig. 1 and Fig. 2). The presence of bla_{NDM-1} explained carbapenem non-susceptibility in 18 strains and the bla_{KPC-3} gene in four strains. Both bioinformatic approaches detected these genes.

Regarding the differences between platforms, EPISEQ® CS identified 21 genomes with at least 1 variant of the *fosA* gene, while the Pathogenwatch and Kleborate platforms identified only 1. Furthermore, EPISEQ® CS did not detect a colistin-resistant strain due to chromosomal mutations detected by the Pathogenwatch platform (Fig. 1 and Fig. 2).

All carbapenem-resistant and carbapenemase-producing *K. pneumoniae* ($n = 22$) carried multiple plasmid replicons according to both approaches. Plasmid incompatibility (Inc) groups IncHI1B (pNDM-MAR) (EPISEQ® CS: 73%, Pathogenwatch + Kleborate: 68%), IncFIB(pNDM-Mar) (EPISEQ® CS: 68%, Pathogenwatch + Kleborate: 50%), and IncC (EPISEQ® CS: 64%, Pathogenwatch + Kleborate: 73%) were the most frequently detected in EPISEQ® CS. All bla_{KPC-3} strains carried IncFIB(K), regardless of the analysis platform (Fig. 1 and Fig. 2).

According to the EPISEQ® CS platform, *K. pneumoniae* strains were clustered into 4 different sequence type (ST)s: 1876 (n = 8), 307 (n = 6), 4839 (n = 5), and 2279 (n = 1). Two genomes were not typified (Supplemental Table 2).

3.3. E. coli

For *E. coli*, the genome size ranged from 4.81–6.27 Mbp (Supplemental Table 2). Both the EPISEQ® CS and BacWGSTdb analyses detected virulence genes associated with adhesion, invasion, iron acquisition through siderophores, genes of the type II secretion system, effectors of the type III secretion system, production of lipopolysaccharide and capsular polysaccharide, outer membrane proteins, production of common *E. coli* pili, toxin secreted from the autotransporter, and genes involved in processes of survival and adaptation to stress (Fig. 3 and Fig. 4). Only the EPISEQ® CS application detected virulence genes involved in secretion systems III, IV, and VI; the broad polar fimbriae; the thermoresistant agglutinin; and colicin A. Only the BacWGSTdb platform detected genes involved in efflux pumps associated with virulence, allantoin production, curli fibers, and the *rcsB* gene, which has multiple functions described (Fig. 3 and Fig. 4).

When analysed by EPISEQ® CS, 20 of 24 (83.3%) strains carried bla_{NDM} (19 bla_{NDM-5} and 1 bla_{NDM-1}), 3 of 24 (12.5%) carried $bla_{OXA-232}$, and 1 (4.2%) carried $bla_{OXA-181}$. In the WGSTdb analysis, similar results were observed, with all bla_{NDM} being typed as bla_{NDM-5}. Genes that confer resistance to aminoglycosides, tetracyclines, sulfonamides, phenicols, trimethoprim, and macrolides were also detected by both platforms, while differences in genes were identified for other classes of antibiotics (Fig. 3 and Fig. 4).

According to the EPISEQ® CS application, 11 of 24 (45.8%) strains were ST2 (Pasteur)-ST167 (Warwick), and 4 of 24 (16.7%) strains were ST650 (Pasteur)-ST 361 (Warwick). In 5 of 24 (20.8%) strains, the ST was not detected (strains 20–2175, 20–853, 20–1202, and 20–2178; Supplemental Table 2). Using the EPISEQ® CS application, serotyping of O and H antigens revealed that 15 of

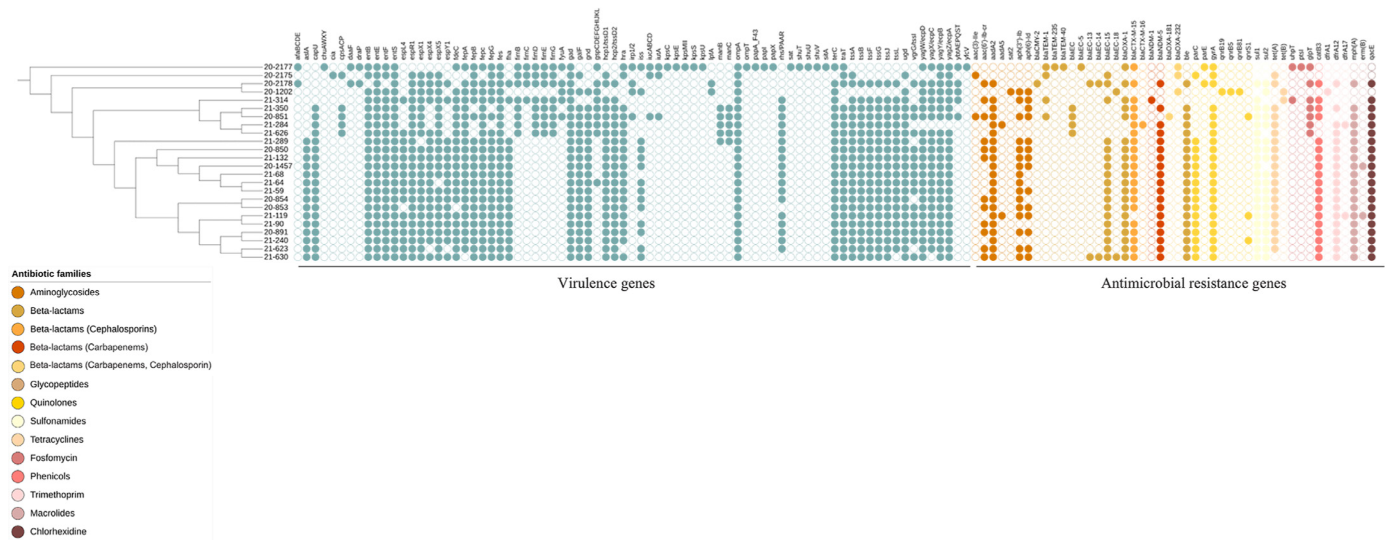

Fig. 3. Distribution of virulence and drug resistance genes in *E. coli* using the EPISEQ CS application.

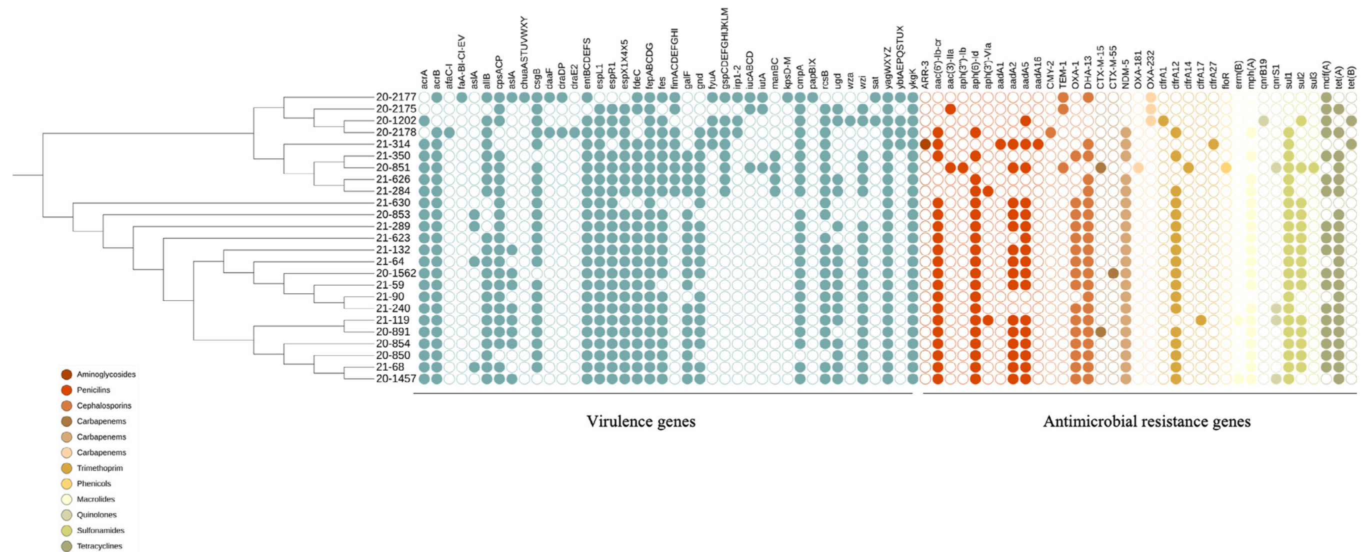

Fig. 4. Distribution of virulence and drug resistance genes in *E. coli* using the BacWGSTdb.

24 (62.5%) strains carried the O101:H10 types and 4 of 24 (16.7%) strains carried the O9:H30 types (Supplemental Table 2).

3.4. A. baumannii

The *A. baumannii* genome size ranged from 3.92–4.09 Mbp (Supplemental Table 2). Regarding the virulome, both platforms detected genes for the production of acinetobactin; Csu pili; poly-β-1,6-N-acetylglucosamine; the outer membrane protein OmpA; and phospholipases C and D, as well as regulation of biofilm production. However, the results obtained using the BacWGSTdb had greater gene coverage, as can be seen when comparing the *bas/bau* genes detected by EPISEQ® CS (*basABCFGHIJ* and *bauABCEF*) with those detected by the BacWGSTdb (*basABCDFGHIJ* and *bauABCDEF*). In addition, the BacWGSTdb detected the *bap*, *adeFGH*, and *abaIFGH* genes (involved in biofilm production), which were not detected by the EPISEQ® CS platform (Fig. 5 and Fig. 6). Neither platform detected genes involved in quorum sensing or the production of type IV pili, capsule or secretion systems, or their respective effectors.

Regarding the resistome, the most frequent carbapenemase-encoding gene detected by both platforms was *bla*$_{\text{OXA-72}}$, followed by *bla*$_{\text{OXA-66}}$ (Fig. 5 and Fig. 6). Both approaches detected a similar number of genes for aminoglycosides, carbapenems (same genes), tetracyclines, phenicols (EPISEQ® CS detected the *floR2* gene for phenicol resistance, and BacWGSTdb did not), and sulfonamides. Additionally, some differences in genes were detected: EPISEQ® CS found the *aph(6′)-Id* gene, and the BacWGSTdb detected the *aac(6′)-Id* gene.

The EPISEQ® CS application detected significantly more penicillin and cephalosporin resistance genes (the majority corresponded to the intrinsic cephalosporin of *A. baumannii*; it also detected chlorhexidine (*qacE*) and quinolone (punctual mutations *parC*, *parE* and *gyrA*) resistance genes that the BacWGSTdb did not. Lastly, both platforms found the same two macrolide resistance genes.

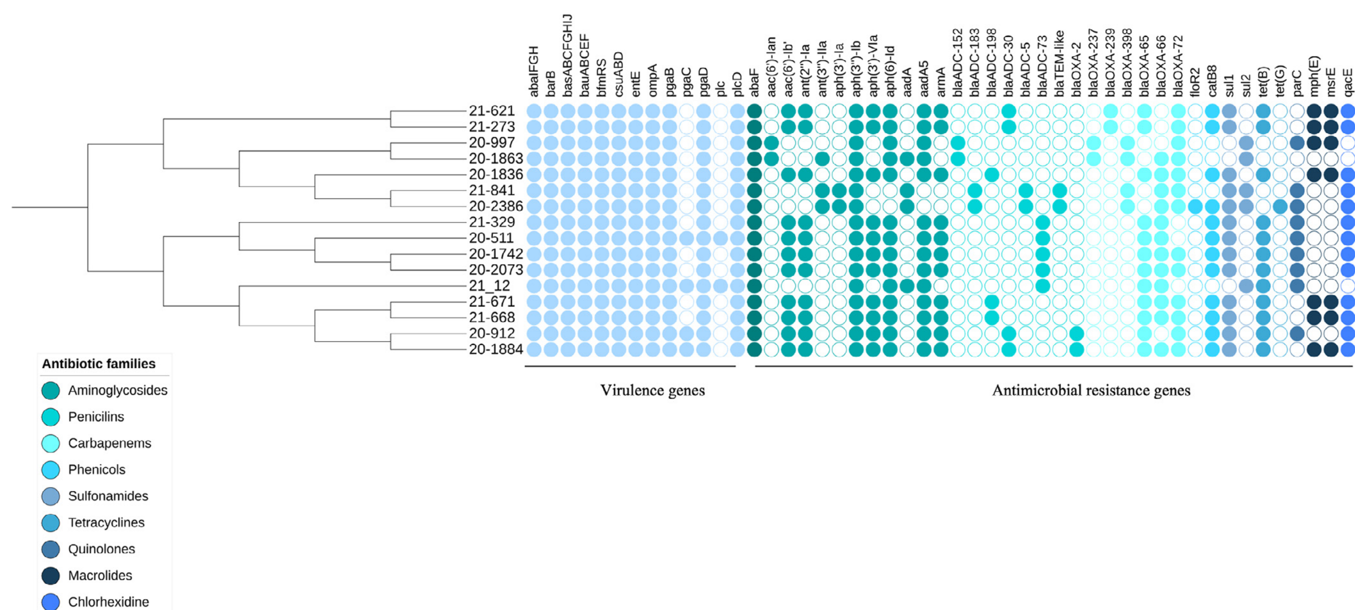

Fig. 5. Distribution of virulence and drug resistance genes in *A. baumannii* using the EPISEQ CS application.

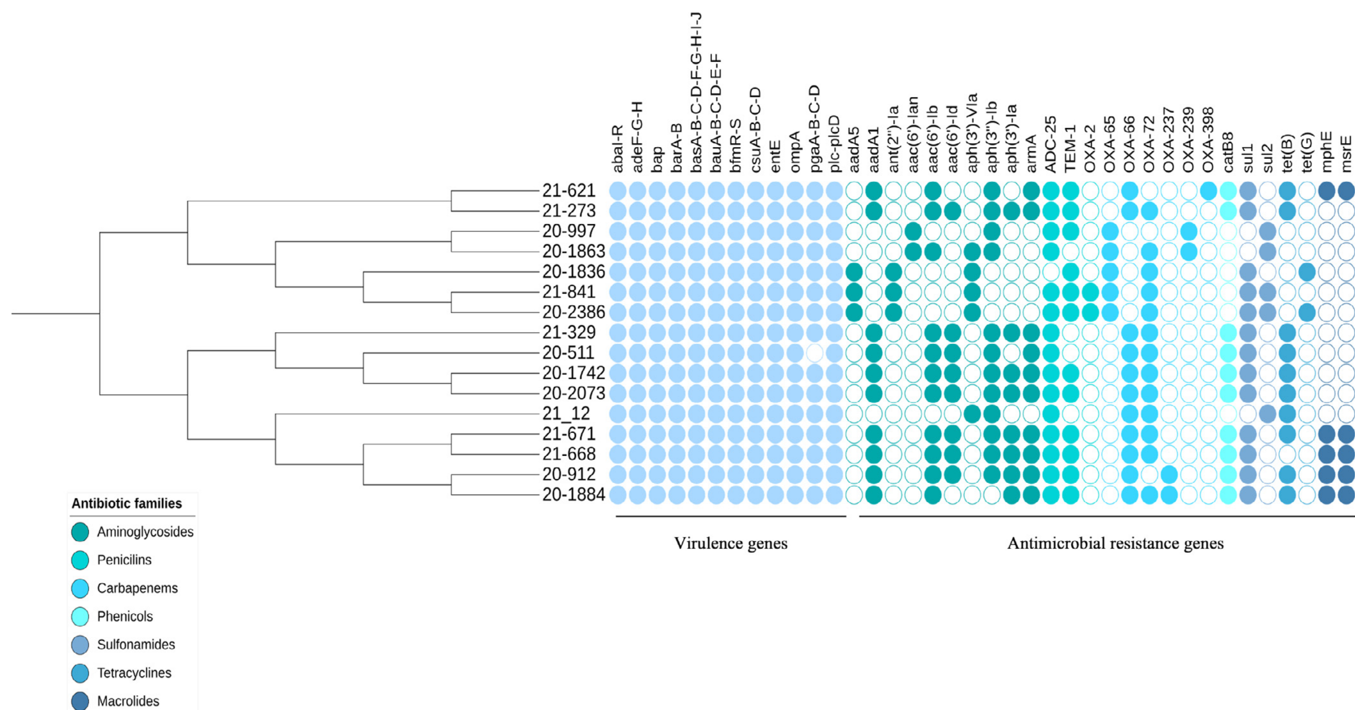

Fig. 6. Distribution of virulence and drug resistance genes in *A. baumannii* using BacWGSTdb platform.

According to the EPISEQ® CS application, five strains were ST208 (Oxford)-ST2 (Pasteur), six strains were ST369 (Oxford)-ST2 (Pasteur), two strains were ST205 (Oxford)-ST156 (Pasteur), and two were ST1694 (Oxford)-ST422 (Pasteur). One strain was ST136 (Oxford)-ST2 (Pasteur) (Supplemental Table 2).

3.5. P. aeruginosa

For *P. aeruginosa*, the genome analysis included only results of the EPISEQ® CS application, as no reliable and curated other bioinformatic tool is available. The genome size ranged from 6.31–7.2 Mb (Supplemental Table 2). The bla_{VIM}, bla_{IMP}, and bla_{GES} genes were detected (Fig. 5), with 8 of 13 (61.5%) strains with no known carbapenemase gene. Multiple virulence genes—mainly those involved in alginate, rhamnolipid, flagella, type IV pili, and lipopolysaccharide production; iron acquisition from the siderophores pyoverdine, pyochelin, and pyocyanin; and the type III and type VI secretion systems and their effectors—were detected in all included strains (Fig. 7 and Fig. 8). Eleven different STs were detected using the Oxford scheme (111, 244, 260, 274, 309 [n = 2], 983, 1487, 2235, 2348, 2731, and 3579). Three strains were not typified (Supplemental Table 2).

3.6. Phylogenetic analysis

Phylogenetic analysis of clinical isolates from each bacterial species was performed, and clusters generated by EPISEQ® CS detected two clusters with 99.64% similarity for *K. pneumoniae*

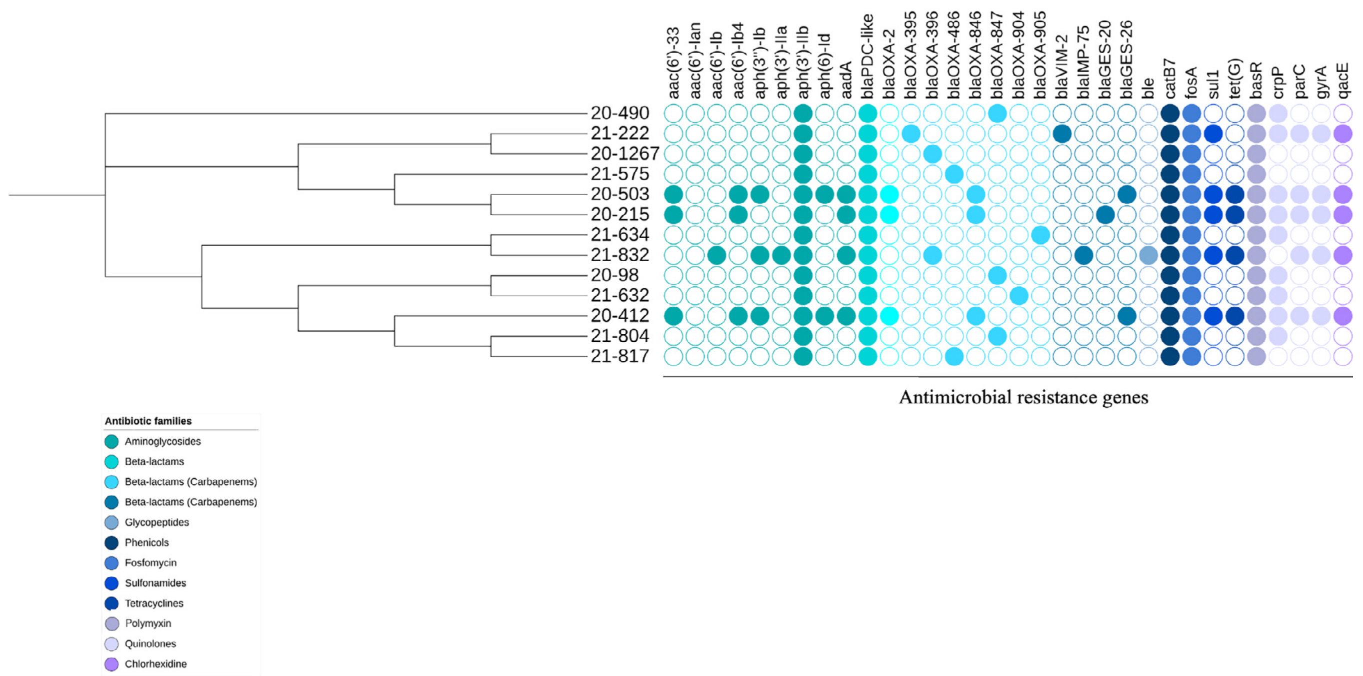

Fig. 7. Distribution of drug resistance genes in *P. aeruginosa* using the the EPISEQ® CS application.

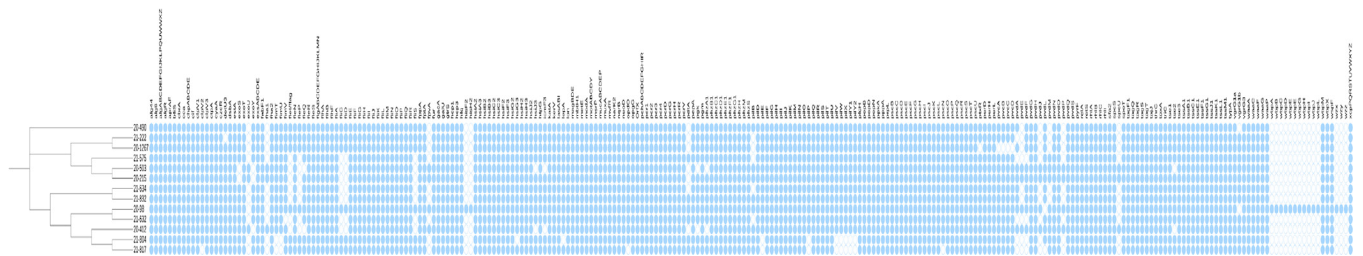

Fig. 8. Distribution of virulence genes in *P. aeruginosa* using the EPISEQ CS application.

(Fig. 9a), one cluster with 97.54 similarity for *E. coli* (Fig. 9b), five clusters with 98.70% similarity for *A. baumannii,* and no clusters were detected with 99.89% similarity for *P. aeruginosa.*

4. Discussion

Pathogen surveillance helps to understand the spread of resistant bacteria. With the use of genomic approaches, there is a growing need for tools that simplify WGS analyses, reducing the need for an expert in bioinformatics and providing results to control the spread of resistant bacteria. Here, we described the use of the web-based software application EPISEQ® CS for genomic analysis of carbapenem non-susceptible Gram-negative bacteria representative form different areas of the country and compared the results with some of the available curated platforms.

For *K. pneumoniae*, the genome size ranged from 5.54–6.62 Mb. In addition to the EPISEQ® CS application, we used the Kleborate platform and the web application Pathogenwatch for bioinformatic analysis [20,21]. Both bioinformatic approaches detected multiple genes encoding aminoglycoside, quinolone, and phenicol resistance.

The most prevalent and highly effective carbapenemase in *K. pneumoniae* is bla_{KPC} [22], and in our study, the most frequent carbapenemase-encoding gene was bla_{NDM-1} (18 of 22, 81.8%), followed by bla_{KPC-3} (4 of 22, 18.2%). The global dissemination of *K. pneumoniae* carrying bla_{NDM-1} is associated with the dissemination of epidemic plasmids. The high prevalence of the bla_{NDM-1} gene renders these infections extremely resistant to treatment [22].

The main differences in genes detected were related to colistin and fosfomycin. Regarding colistin resistance, EPISEQ® CS detected only the plasmid-encoded *mcr1.1* and *mcr9.1* genes, each in only one strain, while Pathogenwatch and Kleborate detected those two genes as well as two strains carrying *mcr1.1* and a chromosomal point mutation in another strain. The *mcr-1* and *mcr-9* colistin resistance genes have been reported as the first and second most commonly detected *mcr* genes worldwide [23]. In the Americas, both the *mcr-1* allele and others have been reported [24]. The EPISEQ® CS application detected 3 different variants of the *fosA* gene (*fosA3, fosA6,* and *fosA7.6*) within 21 of 22 genomes, with some having more than one variant of the gene. Conversely, the Pathogenwatch and Kleborate platforms detected the *fosA* gene with no variant differentiation.

In our study, the most frequently detected ST was ST1876 (n = 8), followed by 307 (n = 6) and 4839 (n = 5). *K. pneumoniae* ST1876 has been scarcely reported, with one strain among 54 (1.85%) detected in China during active surveillance of carbapenem-resistant *Enterobacterales* [25]. In contrast, *K. pneumoniae* ST307 is a potential epidemic clone [26–28], with several outbreak reports [28,29–32], and it has been described as often carrying carbapenem resistance genes, including bla_{KPC-3} and bla_{NDM-1} [26,33].

In *K. pneumoniae,* there are 77 serologically distinct capsular types (1–82), with types 1 and 2 related to severe infections

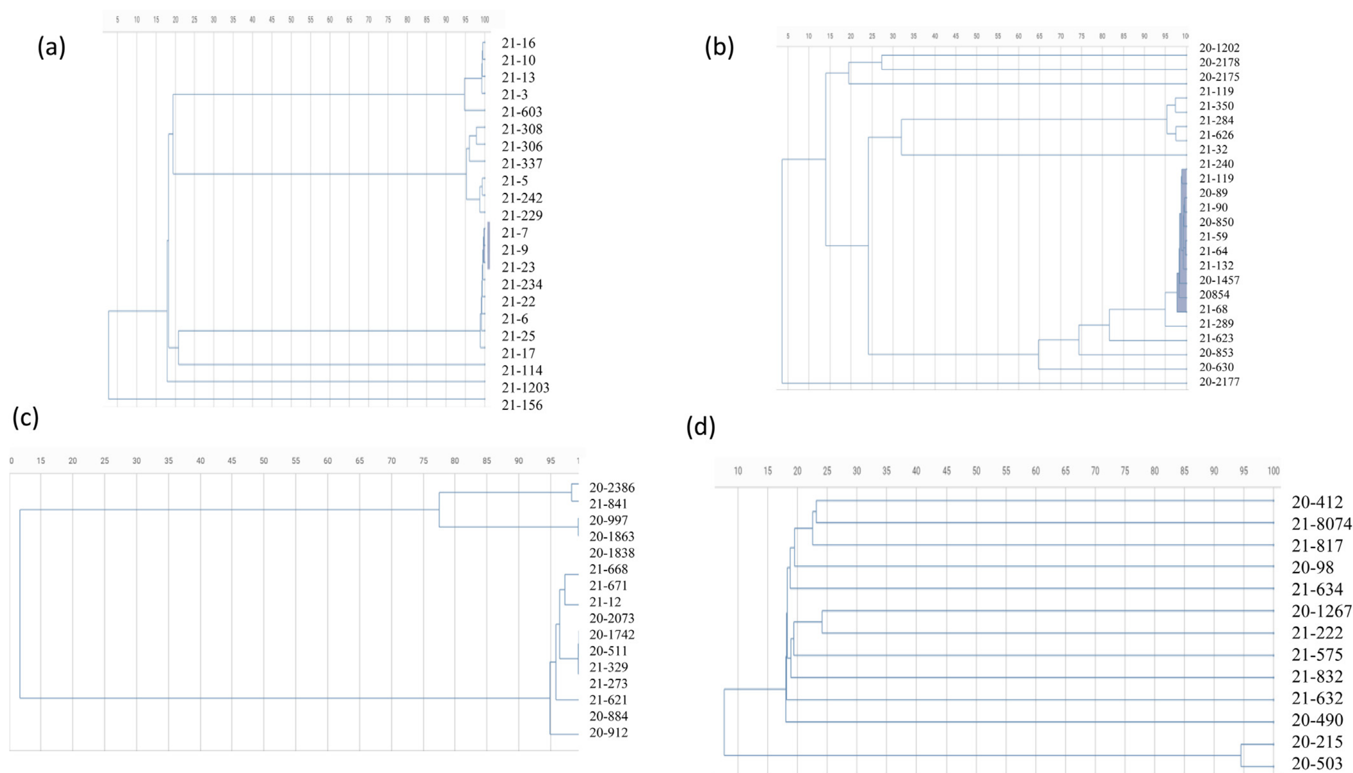

Fig. 9. Clusters generated by EPISEQ CS. (a) *K. pneumoniae* clusters with 99.64% of similarity; (b) *E.* coli cluster with 97.54% similarity; (c) *A. baumannii* with 98.70% similarity; and (d) *P. aeruginosa* with nether similarity.

caused by hypervirulent strains [34]. WGS-based approaches have provided significant insights into the genetic variability of the capsular locus of *K. pneumoniae*, identifying 134 capsular types, but genotypic-biochemical capsular type correlations are still lacking [35]. Our study detected capsular type 15 (9 of 22, 40.9%) strains, followed by type 102 (6 of 22, 27.3%). There are few reports worldwide of these capsular types [36].

Unlike other pathogens, *K. pneumoniae* has several platforms for analysing genomes, for instance, Kleborate, Pathogenwatch, BacWGSTdb, Virulence Factor Database, and is included in the EPISEC framework. However, Kleborate was designed to perform a comprehensive and deep analysis that the other platforms do not perform; for example, capsule and o-antigen typing, the search of capsule regulators that are important drivers of hypervirulence, the assignation of variants of integrative conjugative elements, plus the characterization of acquired and chromosomal antimicrobial resistance genes.

The genome size of *E. coli* is usually reported to be between 4.5 and 5.5 Mbp, with some projects even considering sizes around 6 Mbp as part of the range [37]. Most of our strains match this range. For *E. coli*, EPISEQ® CS classified the β-lactam resistance genes into more specific categories according to the class of β-lactams compared with BacWGSTdb. Likewise, EPISEQ® CS detected a considerable number of quinolone resistance genes and point mutations—such as *gyrA, parC,* and *parE*—which were not reflected in the BacWGSTdb results. In contrast, BacWGSTdb detected the *ARR-3* rifampin resistance gene and a greater number of aminoglycoside, trimethoprim, sulfonamide, and tetracycline resistance genes than EPISEQ® CS.

In a recent study, 229 carbapenemase-producing *E. coli* strains from 36 countries (including 6 strains from Mexico) were analysed in 2015–2017, and the most frequent carbapenemase encoding genes were $bla_{OXA\text{-}181}$ (23%), followed by $bla_{NDM\text{-}5}$ (20%), $bla_{OXA\text{-}48}$ (17%), bla_{KPC} (15%), and $bla_{NDM\text{-}1}$ (10%) [38]. On the contrary, in our study (n = 25), most strains carried $bla_{NDM\text{-}5}$ (80%), followed by $bla_{OXA\text{-}232}$ (12%) and $bla_{NDM\text{-}1}$ (4%). Only one strain carried $bla_{OXA\text{-}181}$ (4%). These differences may be explained by the number of strains analysed and the different variety of locations included in both studies.

For *E. coli* and according to the EPISEQ® CS application, 12 of 25 (48%) strains were ST2 (Pasteur)-ST167 (Warwick). This ST has been reported in healthy dogs in France in two highly predominant plasmids (*bla*CTX-M-1/IncI1/ST3 and *bla*CMY-2/IncI1/ST2) [39] and in *E. coli* O157 isolates collected from humans in the United States in 2000–2008. In that study, the predominant ST was ST2 (5 of 33) [40].

Using the EPISEQ® CS, serotyping in *E. coli* of O and H antigens revealed that 16 of 25 (64%) strains carried the O101:H10 types. This serotype has been reported previously in Spain [41] with very low prevalence.

Regarding *A. baumannii*, our results (genome range: 3.92–4.09 Mb) match the expected genome size of this species, as reported in previous studies (maximum expected genome size: 4.4 Mbp) [42]. The most frequent carbapenemase encoding gene detected was $bla_{OXA\text{-}72}$ ($bla_{OXA\text{-}24/40}$-like), followed by $bla_{OXA\text{-}66}$. Other detected bla_{oxa} genes were $bla_{OXA\text{-}2}$, $bla_{OXA\text{-}65}$, $bla_{OXA\text{-}237}$, $bla_{OXA\text{-}239}$ ($bla_{OXA\text{-}23\text{-}like}$), and $bla_{OXA\text{-}398}$ (Fig. 5 and Fig. 6). The most frequent bla_{OXA} genes previously detected in *A. baumannii* in Mexico are $bla_{OXA\text{-}24/40}$-like, followed by $bla_{OXA\text{-}23}$-like [43].

Something to note about the virulome analysis of *A. baumannii* strains is that genes involved in characteristic virulence factors of this species—such as type IV pili, capsular polysaccharide, type II and VI secretion systems, and lipooligosaccharide—were not detected by either platform. Type IV pili genes are fundamental in the twitching motility of *A. baumannii* and are rarely absent in *A. baumannii* genomes [44]. Interestingly, a wide variety of type IV pili were detected in the virulome of *P. aeruginosa* strains by EPISEQ® CS, meaning that this area of opportunity is exclu-

sive to *A. baumannii*. The capsular polysaccharide has even been named the "main virulence factor" of this species [45], as its structure determines the virulence of a strain to such a degree that strains lacking the capsule are considered avirulent [46,47]. Bioinformatic tools such as Kaptive have been recently optimized to typify *A. baumannii* strains according to their K (capsular) and OC (oligosaccharide) locus [48]. A virulome analysis of this species must be deep enough to cover at least these virulence factors for better surveillance of potentially hypervirulent, multidrug-resistant strains.

A. baumannii has been studied using two MLST schemes, the Oxford [49] and the Pasteur [50]. We used the Pasteur scheme for epidemiological comparison because it has been reported to be adequate for epidemiological studies [51].

In our study, 12 of 16 strains were detected to be of the ST2 (Pasteur) scheme. *A. baumannii* ST2 has been reported to be predominant in Germany [52], Pakistan, and Iran [53,54], and was also detected as the predominant ST in a previous study at a tertiary-level hospital in Mexico [55]. In our study, the ST136 was detected in one clinical isolate. This ST has been reported as a high-risk persistent clone involved in an outbreak [56].

The range of genome size (6.31–7.2 Mb) of *P. aeruginosa* strains matched what has been reported previously for this species [57]. *P. aeruginosa* is intrinsically resistant to ertapenem, and a large number of isolates are resistant to other carbapenems by the loss of OprD porin (imipenem) or in combination with upregulation of MexAB-OprM efflux pump (meropenem and doripenem) [58]. In our study, most strains carried no class A or B carbapenemase-encoding genes but carried class D oxacillinase-encoding genes (bla_{OXA}) associated with carbapenem resistance by multiple databases such as the Comprehensive Antibiotic Resistance Database and ResFinder [59,60], and these genes may contribute to carbapenem resistance. In this bacterial species, the most frequently detected genes associated with carbapenem resistance in Mexico were found (bla_{VIM}, bla_{IMP}, and bla_{GES}); both additional carbapenemases have been detected on the same continent. For example, in the United States, carbapenemase-encoding genes detected in *P. aeruginosa* include *bla*KPC, *bla*NDM, *bla*VIM, and *bla*IMP [61,62]; the same genes have been detected in Latin America, plus bla_{GES} and bla_{SPM} [63]. Multiple virulence genes were detected in all *P. aeruginosa* included strains (Fig. 7 and Fig. 8). Among the 13 strains sequenced, 11 different STs were detected using the Oxford scheme (ST111, ST244, ST260, ST274, ST309 [n = 2], ST983, ST1487, ST2235, ST2348, ST2731, and ST3579). It has been described that *P. aeruginosa* has a nonclonal and epidemic structure, and for this reason, attempts to infer the relationships between strains are meaningless, except the most related groups. Additionally, a wide diversity of STs has been reported, with distribution in both clinical and environmental sources [64,65].

There are no curated bioinformatics platforms to determine the genomic characteristics and molecular epidemiology of *P. aeruginosa*; the unique option was EPISEQ® CS, which demonstrated that a vast majority of the virulence genes of this species were registered in the Virulence Factor Database [66] and were non-registered genes. Some of these are putative, such as *kerV* and *spcU*, or metabolism-involved genes, such as *metE, pgk, purD,* and *pyrF*, for which very scarce information can be found that associates them with *P. aeruginosa* virulence.

There are limited reports about the use of the EPISEQ® CS application. A previous report on EPISEQ® CS solution explored the diversity of *Staphylococcus aureus* in neonatal bloodstream infections. In that study, the use of the EPISEQ® CS application to assess the diversity allowed ruling out an outbreak [67]. An easy-to-use web-based application (EPISEQ® SARS-CoV-2) to analyse SARS-CoV-2 next-generation sequencing revealed 100% concordance with reference methods in clade and lineage classification [68]. Thus, EPISEQ® SARS-CoV-2 has been proven to be a reliable analysis of raw next-generation sequencing to support genomic surveillance of SARS-CoV-2.

In our study, for EPISEQ® CS analysis, once the FASTQ was obtained, the bioinformatic analysis was conducted by a one-click instruction, and ca. 15 min were needed for complete genome analysis. This software is designed for a list of bacterial species (n = 14) covering the majority of HAI-related organisms, and only FASTQ data generated by Illumina are accepted for analysis. As WGS becomes more affordable and applicable to routine epidemiological surveillance, EPISEQ® CS may be a helpful tool for monitoring the spread of drug-resistant bacteria.

This study has some limitations, e.g. it presents mainly genomic information, especially in the virulome, regardless of phenotype; it only includes four bacterial species, and other species of clinical relevance are not considered, especially *S. aureus,* which is part of the list of species offered by EPISEQ® CS.

5. Conclusion

EPISEQ® CS, compared with the other available platforms, enabled a comprehensive resistance and virulence analysis, providing a reliable method for bacterial strain typing and characterization of the virulome and resistome, plus MLST analysis.

Competing interests

None to declare.

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Ethical Approval

The local ethics committee of Hospital Civil de Guadalajara Fray Antonio Alcalde (Jalisco, Mexico) approved this study (reference number 129/17). The ethics committee waived informed consent because no intervention was involved, and no patient identifying information was included. All participating institutions agreed with the present study.

Data availability

The genomic sequence data were submitted to the National Center for Biotechnology Information (BioProject accession no. in Process).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.02.026.

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