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Molecular characterization of carbapenemase-producing Enterobacterales in a tertiary hospital in Lima, Peru

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ABSTRACT Carbapenemase-producing Enterobacterales (CPE) are a growing threat to global health and the economy. Understanding the interactions between resistance and virulence mechanisms of CPE is crucial for managing difficult-to-treat infections and informing outbreak prevention and control programs. Here, we report the characterization of 21 consecutive, unique clinical isolates of CPE collected in 2018 at a tertiary hospital in Lima, Peru. Isolates were characterized by phenotypic antimicrobial susceptibility testing and whole-genome sequencing to identify resistance determinants and virulence factors. Seven *Klebsiella pneumoniae* isolates were classified as extensively drug-resistant. The remaining *Klebsiella*, *Enterobacter hormaechei,* and *Escherichia coli* isolates were multidrug-resistant. Eighteen strains carried the metallo-β-lactamase NDM-1, two the serine-carbapenemase KPC-2, and one isolate had both carbapenemases. The bla_{NDM-1} gene was located in the truncated ΔISAba125 element, and the bla_{KPC-2} gene was in the Tn4401a transposon. ST147 was the most frequent sequence type among *K. pneumoniae* isolates. Our findings highlight the urgent need to address the emergence of CPE and strengthen control measures and antibiotic stewardship programs in low- and middle-income settings.

IMPORTANCE Genomic surveillance of antimicrobial resistance contributes to monitoring the spread of resistance and informs treatment and prevention strategies. We characterized 21 carbapenemase-producing Enterobacterales collected at a Peruvian tertiary hospital in 2018, which exhibited very high levels of resistance and carried numerous resistance genes. We detected the coexistence of carbapenemase-encoding genes (bla_{NDM-1} and bla_{KPC-2}) in a *Klebsiella pneumoniae* isolate that also had the PmrB(R256G) mutation associated with colistin resistance. The bla_{KPC-2} genes were located in Tn4401a transposons, while the bla_{NDM-1} genes were in the genetic structure Tn125 (ΔISAba125). The presence of high-risk clones among *Klebsiella pneumoniae* (ST11 and ST147) and *Escherichia coli* (ST410) isolates is also reported. The study reveals the emergence of highly resistant bacteria in a Peruvian hospital, which could compromise the effectiveness of current treatments and control.

KEYWORDS carbapenems, carbapenem-producing Enterobacterales, drug resistance, Whole-genome sequencing, Peru

A ntimicrobial resistance (AMR) poses a significant threat to human health and the global economy. The rise of multidrug-resistant (MDR) and pan-resistant (PDR) bacteria jeopardizes the advancements in modern medicine, potentially leading us toward a post-antibiotic era (1). Carbapenems, vital antibiotics often reserved for severe infections caused by MDR Gram-negative bacilli, are now threatened by the emergence of carbapenemase-producing Enterobacterales (CPE) (2). These pathogens are notably

difficult to treat due to the limited effectiveness of available drugs, forcing clinicians to revert to older medications such as colistin (2, 3).

Carbapenemases, as categorized by Ambler's classification, include Class A *Klebsiella pneumoniae* carbapenemase (KPC), Class B metallo-β-lactamases (MBLs) like New Delhi MBL (NDM), and Class D oxacillinases (OXA) such as OXA-48-like enzymes found in Enterobacterales (2). *Klebsiella pneumoniae* and *Escherichia coli*, among the CPE, have emerged as significant global health threats due to their high mortality rates (4). The widespread dissemination of CPE is primarily attributed to the horizontal transfer of antibiotic-resistance genes via mobile genetic elements, including plasmids and transposons (5). Various virulence factors enhance the pathogenicity of CPE. While there is extensive knowledge about the interplay between these factors and AMR in many bacterial pathogens (6), the specific genetic determinants that drive virulence and their interaction with resistance mechanisms are not fully understood (7).

In Peru, the first CPE infections were identified in late 2013 as a carbapenem-resistant *K. pneumoniae*, sequence type (ST) 340 of the high-risk clonal complex (CC) 258 (8, 9). Subsequent reports indicate the spread of various carbapenemases in clinical settings and non-traditional hosts such as *Providencia stuartii* (10, 11). By 2020, the spread of the metallo-β-lactamase NDM-1 in *K. pneumoniae* was evident, involving newer lineages like ST348 and ST147 (12). Despite numerous CPE reports in Peru and Latin America, comprehensive genomic data are needed to conduct in-depth evolutionary, molecular, and epidemiological studies. Understanding the relationships between different CPE strains, both locally and globally, is essential. To address this gap, we sequenced the genomes and analyzed the resistance and virulence determinants of 21 CPE samples from a tertiary hospital in Lima collected in 2018.

RESULTS

Antimicrobial susceptibility profiles

The results of antibiotic susceptibility testing of 12 *K. pneumoniae*, 1 *Klebsiella quasipneumoniae*, 2 *Klebsiella aerogenes*, 2 *E. coli*, and 4 *Enterobacter hormaechei* are shown in Fig. 1. Overall, all CPE isolates were resistant to penicillins plus β-lactamase inhibitors, cephalosporins, monobactams, and fluoroquinolones. Furthermore, nearly all CPE isolates were resistant to phenicols (19/21, 90.5%) and folate pathway inhibitors (18/21, 85.7%). All *Klebsiella* species were resistant to gentamicin, and almost all were susceptible to amikacin, whereas all *E. hormaechei* were resistant to amikacin but mainly susceptible to gentamicin. *E. coli* isolates were predominantly susceptible to both aminoglycosides. Resistance to fosfomycin (10/12, 83.3%) and colistin (10/12, 83.3%) was observed only among *K. pneumoniae* isolates, whereas the other CPE were susceptible to both drugs. Phenotypic screening of carbapenemase production resulted in the identification of 19 metallo-β-lactamase-producers and 3 serine-carbapenemase producers. Notably, strain KpCR02 tested positive for serine and metallo-carbapenemase enzymes using phenotypic methods. Seven *K. pneumoniae* strains were classified as extensively drug-resistant (XDR), and the remaining CPE as MDR (Fig. 1; Table S1).

Genomic diversity

Four STs were identified among *K. pneumoniae* isolates: ST11 ($n = 1$), ST147 ($n = 8$), ST348 ($n = 1$), and ST4872 ($n = 2$) (Fig. 2). Among the *K. pneumoniae* ST147 genomes, they diverged by 21–115 single nucleotide polymorphisms (SNP). The *K. quasipneumoniae* isolate was classified as ST4952, and both *K. aerogenes* isolates were ST215. ST88 and ST471 were identified among *E. coli* isolates. The four *E. hormaechei* isolates were classified as ST2054.

Antimicrobial resistance genes

K. pneumoniae isolates harbored more AMR genes (average = 16.8, SD = 3.3) than other species in this study (average = 14.0, SD = 3.4). The bla_{OXA-1} gene was detected in
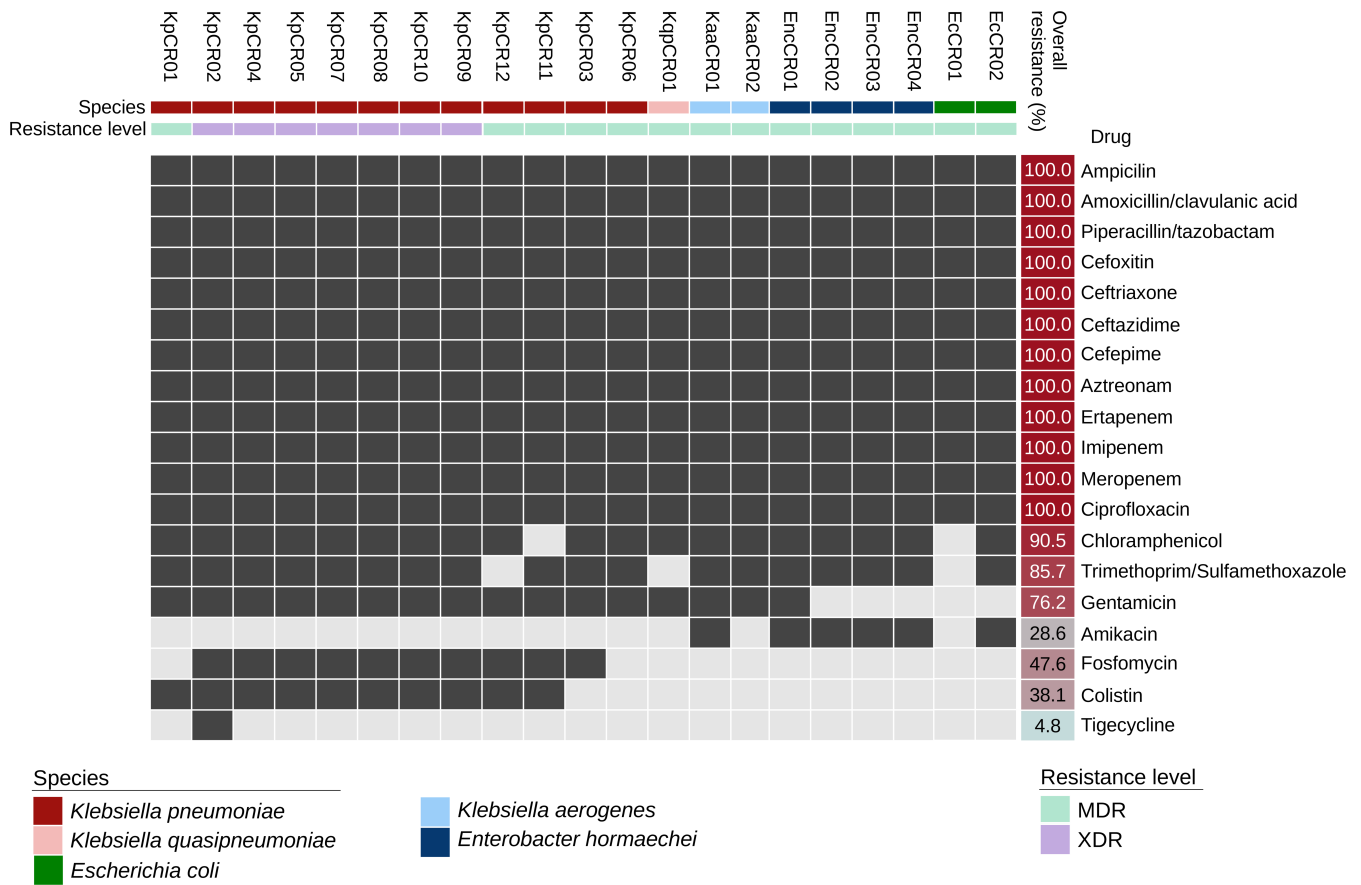
FIG 1 Antimicrobial susceptibility profiles of Enterobacterales by antimicrobial category. Black and gray boxes indicate resistance and susceptibility, respectively.

all CPE isolates, while *sul1* and *arr*-3 (20/21, 95.2%), *catB3* (19/21, 90.5%), and *aac(6′)-lb-cr5* (18/21, 85.7%) genes were detected in nearly all isolates. Several AMR genes responsible for resistance to non-carbapenem β-lactams, including AmpC β-lactamases, extended-spectrum β-lactamases (ESBLs), aminoglycosides, fluoroquinolones, and other antimicrobial classes, were also detected among CPE isolates. Regarding carbapenemase-encoding genes, bla_{NDM-1} (19/21, 90.5%) was detected in almost all isolates, whereas bla_{KPC-2} (3/21, 14.3%) was detected in two *K. pneumoniae* and one *E. coli*. The *K. pneumoniae* strain KpCR02 tested positive for both bla_{NDM-1} and bla_{KPC-2} genes.

Genetic context of carbapenemase genes

The bla_{KPC-2} gene was found in *K. pneumoniae* KpCR01 and KpCR02, and *E. coli* EcCR01, located in a Tn4401a transposon of the Tn3 family. The gene was flanked by ISKpn6 and ISKpn7 elements near the *tnpA* and *tnpR* transposase genes (Fig. 3A). The bla_{NDM-1} gene in *K. pneumoniae* strain KpCR09, *E. hormaechei* strain EnCCR02, and *E. coli* strain EcCR02 was found within the ΔTn125 element (Fig. 3B). The bla_{NDM-1} gene was flanked by ISA-ba125 and the *blaMBL* gene (Fig. 3B) in both reference strains and the three isolates described here. In *K. pneumoniae* strain KpCR09, the *blaTEM-26* and *aac (3)-lle* genes, which encode resistance to third- and fourth-generation cephalosporin and aminoglycoside-modifying enzyme (AME), respectively, were identified upstream of ΔTn125.

Virulence factors

K. pneumoniae isolate KpCR01 presented the yersiniabactin *ybt9* gene located on the integrative conjugative element (ICE) Kp3, along with the genes encoding for the *wzi50* capsular type and antigenic determinants K15 and O4. In contrast, isolate KpCR11
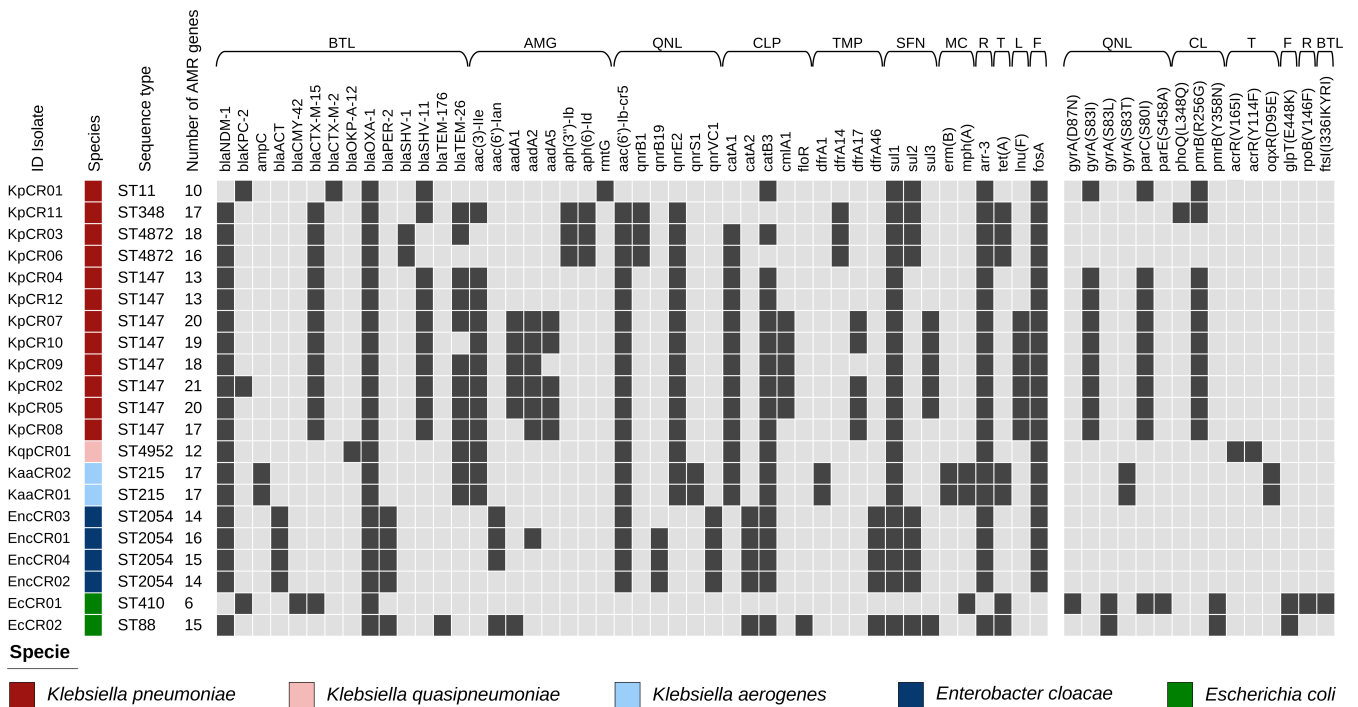
FIG 2 Resistance genes of carbapenem-resistant Enterobacterales. Total AMR genes are summarized by bacterial isolate, and individual annotated genes conferring resistance are also summarized by antimicrobial category. BTL, β-lactams; AMG, aminoglycoside; QNL, quinolone; CLP, chloramphenicol; TMP, trimethoprim; SFN, sulfonamide; MC, macrolide; R, rifamycin; T, tetracycline; L, lincosamide; CL, colistin; and F, florfenicol.

exhibited the *ybt0* gene within the ICEKp12 element, accompanied by the *wzi94* capsular type gene and antigenic determinants K62 and O1. Notably, all *K. pneumoniae* ST147 isolates in our study had the aerobactin (*iuc5*) gene, genes encoding for the *wzi64* capsular type, and antigenic determinants KL64 and O2a. Additionally, in our two *E. coli* isolates, we identified several virulence genes, including *fimH*, associated with type 1 fimbriae; *iucC*, related to aerobactin synthetase; *iutA*, encoding the ferric aerobactin receptor; *lpfA*, indicative of long polar fimbriae; and *sitA*, associated with iron acquisition (Table S2).

DISCUSSION

The rapid spread of CPE poses a significant threat to global public health. Our study of CPE from one Peruvian hospital in 2018 confirms the increasing prevalence of bla_{NDM-1} over bla_{KPC-2} in Peru since 2013, alongside the emergence of OXA-48-like carbapenemases, particularly OXA-181 (3–5, 13).

Our findings confirm high resistance levels in CPE, particularly in *Klebsiella pneumoniae*. Notably, the KpCR02 isolate exhibited up to 21 AMR genes, including NDM-1 and KPC-2. All NDM-1-producing CPE showed resistance to β-lactams, including aztreonam, often due to AmpC and ESBL genes, with the blaCTX-M-15 gene being particularly prevalent in our data set (14, 15). The co-production of carbapenemases NDM-1 and KPC-2 in *K. pneumoniae* has been previously described in South America, including Brazil, Argentina, Uruguay, Ecuador, and Paraguay (16). Our report provides further evidence of this occurrence and regional dispersion of CPE co-producing NDM-1 and KPC-2 carbapenemases.

The aminoglycoside resistance observed in our isolates could be attributed to genes that encode AMEs. The *rmtG* gene involved in 16S rRNA methylation confers high-level resistance to aminoglycosides (minimum inhibitory concentration, MIC ≥ 256 µg/mL) and is widely spread in *K. pneumoniae* found in South America (17). Among the AMEs, the acetyltransferases aac(3)-IIe and aac(6′)Ib-cr5 were the most frequently detected. The
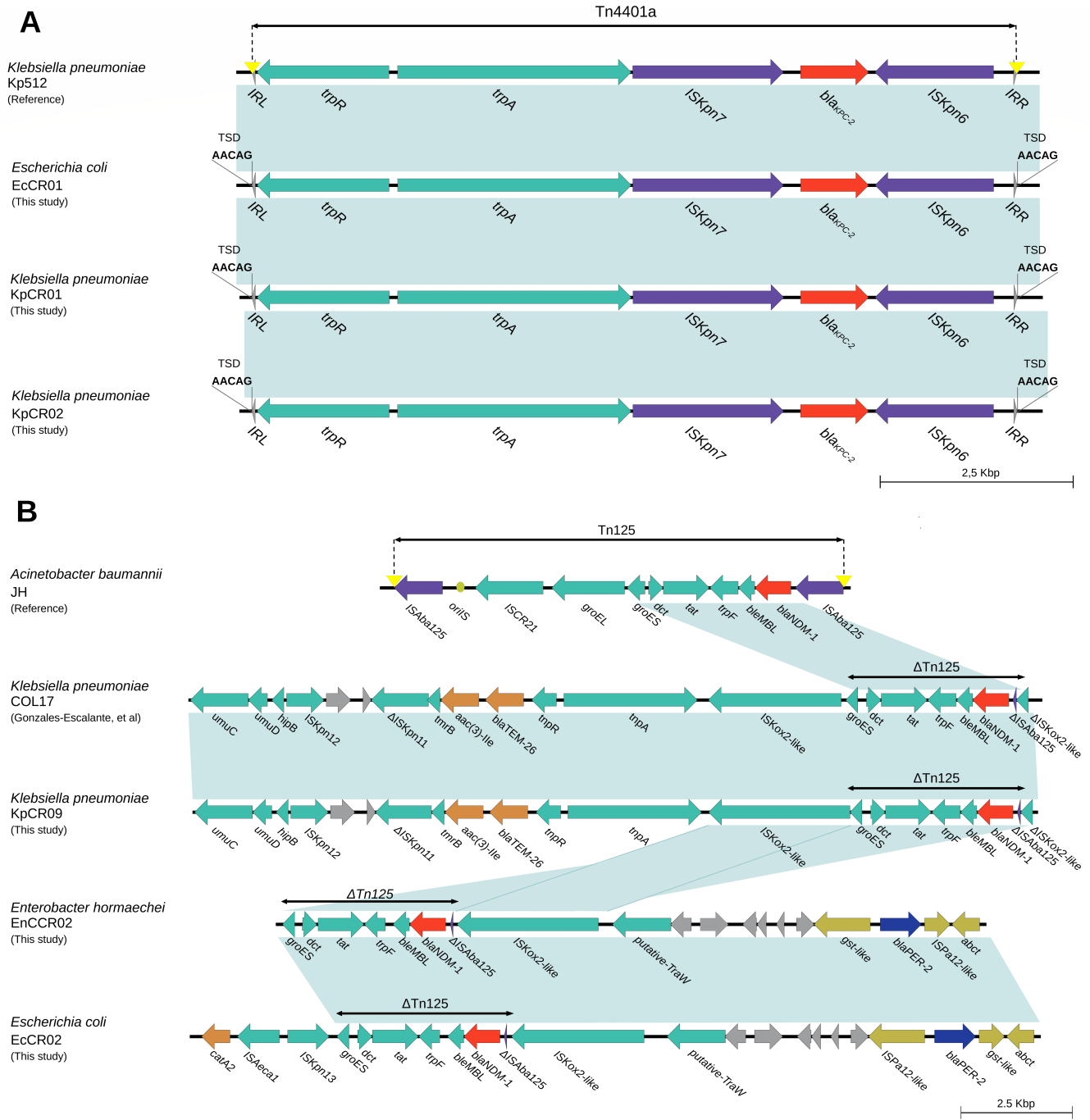
FIG 3 (A) Genetic context of the structures of the Tn4401a transposon carrying bla_{KPC-2} (GenBank accession number KT378596) and (B) genetic context of Tn125 (GenBank reference number JN872328) in our Enterobacterales isolates. Shading indicates 100% sequence similarity.

aac (3)-IIa has been associated with resistance to gentamicin, tobramycin, and netilmicin, as described elsewhere (18). The aac(6')Ib-cr is characterized by W102R and D179Y/G substitutions [both substitutions being present in aac(6')Ib-cr5], which, in addition to its capacity to inactivate several aminoglycosides, also result in the ability to inactivate several fluoroquinolones such as ciprofloxacin and norfloxacin (19). Nine *K. pneumoniae* and all *E. coli* isolates had amino acid substitutions at GyrA and ParC, the most common quinolone resistance mechanism (20). The remaining CPE did not present mutations in the established quinolone targets. However, all presented at least two transferable

mechanisms of quinolone resistance and alterations in efflux pump regulators in one case. While highly unusual several years ago, this scenario is increasingly being described (19, 20).

Resistance to colistin observed in *K. pneumoniae* isolates could be explained by the amino acid substitution detected in the PmrB (R256G) of 10 isolates, one of which also had the PhoQ(L348Q) mutation. Remarkably, both mutations are associated with lipid A modification (21). PmrB amino acid substitution (Y358N) was detected in two *E. coli* isolates; however, both were susceptible to colistin. Although the polymorphism may not be ruled out, this amino acid substitution has been previously described in *E. coli* isolates with high levels of colistin resistance (MIC >32 µg/mL) in the absence of *mcr* genes or other alteration in PmrAB, PhoPQ, or MgrB. Therefore, this single substitution may play a role in the development of colistin resistance (22).

Our genomic analysis found ST147 to be the dominant clone in *K. pneumoniae*, known for its high drug resistance and global dissemination. This includes the KpCR02 isolate, exhibiting both KPC-2 and NDM-1 and colistin resistance through pmrB mutations. The global emergence of KPC has been influenced by the wide dispersion of *K. pneumoniae* CC258, including ST11 and ST340 (23). ST11 is endemic in Brazil and several European countries (24, 25), and ST340 has been previously reported in Peru (7). Several *K. pneumoniae* ST11, ST147, and ST348 outbreaks have also been reported in Peru (10, 26). Our report of *K. pneumoniae* KPC-producing ST11 adds evidence of a high-risk international clone in Peru. In contrast to other reports, *K. pneumoniae* ST348 isolate KpCR11 described in this study significantly differs from other ST348 strains characterized by CTX-M-15 and KPC-3 production (27). The study reports *K. pneumoniae* ST4872, *K. quasipneumoniae* ST4952, and *K. aerogenes* ST215 for the first time in Peru. Among *E. coli* strains, ST471 and ST88 were identified, known for their resistance and virulence, alongside the first reported cases of *E. hormaechei* ST2054 in the region.

Analysis of the genetic context of the bla_{KPC-2} gene revealed that it was located in the Tn4401a transposon, a member of the Tn3 transposon family (28, 29). To our knowledge, this is one of the few reports on Tn4401a in *E. coli* (30). Of note, current data differ from the previous genetic environment of bla_{KPC-2} detected in Peru, highlighting the presence of different genetic structures carrying this gene in the country (7). Furthermore, the analysis of the genetic context of the bla_{NDM-1} gene revealed that it was located in a truncated structure of Tn125. The architecture of the characterized ΔTn was identical to those previously reported in Peru and other Latin American countries (31, 32), and no differences were observed among the other CPE described here.

Hypervirulence is associated with the presence of additional siderophores, yersinia-bactin (*ybt*), aerobactin (*iuc*), salmochelin (*iro*), and specific capsular serotypes (K1, K2, and K5) (33). Interestingly, the aerobactin *iuc5* gene was detected in all *K. pneumoniae* ST147 samples. Besides potentially playing a critical role *in vitro* and *in vivo* virulence, it is a marker to identify highly virulent strains (33). This finding is compatible with that described in other CC258 isolates (34); however, in our isolate, neither characteristic virulence determinants such as salmochelin or colibactin nor genetic determinants associated with hypermucoviscosity (*rmpA*) were detected. To date, 12 distinct O loci have been identified, with O1 and O2 as the most common antigens (35), while K-type variation has been associated with six conserved genes (*galF, orf2, wzi, wza, wzb,* and *wzc*). The *wzi* gene best predicts K antigen-related virulence (36).

The study's limitations include a need for gene expression data and a limited sampling period from one healthcare center in 2018, which restricts the broader application of our findings. Instead, the results described here should be considered reference data and a starting point for further studies using more complex designs and sampling methods.

Overall, our comprehensive analysis of 21 CPE isolates underscores the significant challenge posed by drug-resistant CPE strains, emphasizing the need for expanded genomic surveillance, effective infection control, and antimicrobial stewardship

programs to curb the spread of CPE and maintain the efficacy of antimicrobial agents, particularly in the understudied Latin American region.

MATERIALS AND METHODS

Clinical isolates

From January to December 2018, 21 consecutive, non-replicated, and unique suspected CPE isolates were chosen based on their phenotypic resistance to meropenem at an MIC of ≥1 µg/mL using disk-diffusion assays. Clinical isolates were obtained from blood, urine, and other biological samples from inpatients and outpatients admitted to the tertiary public hospital, Hospital Nacional Arzobispo Loayza in Lima, Peru. The hospital's laboratory characterized all isolates at the species level using a standard biochemical test panel (37) and sent them to Universidad Peruana Cayetano Heredia (UPCH) for phenotypic and genomic characterization.

Carbapenemase screening and identification

The phenotypic screening of carbapenemase production was done with the Triton Hodge Test and the carbapenem inactivation method as previously described (38). Inhibition tests using EDTA and phenylboronic acid were used to distinguish classes of carbapenemases (39).

Antimicrobial susceptibility testing

Resistance phenotypes were determined using the VITEK2 System with AST-N249 cards (bioMérieux, France) following the manufacturer's instructions. MIC values were interpreted using clinical breakpoints established by the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines (40). The European Committee for Antimicrobial Susceptibility Testing breakpoints were used for tigecycline interpretation since CLSI breakpoints were not available at the time of the study (41). Colistin susceptibility was assessed by the colistin broth disk elution test as previously described (42). Intermediate resistance results were analyzed and reported as resistant, and the criteria suggested by Magiorakos et al. were used to classify bacteria as MDR, XDR, or PDR (43).

DNA extraction and whole-genome sequencing

Genomic DNA was extracted using the GeneJET DNA purification kit (Thermo Fisher Scientific, USA) from single colonies that were incubated in 1 mL of tryptic soy broth (Becton & Dickinson, USA) at 37°C and shaking at 300 rpm for 8 hours. The extracted gDNA was quantified with a Qubit 4.0 fluorometer, and Illumina libraries were constructed using 1 ng of gDNA with the Nextera XT kit (Illumina, USA). Paired-end libraries were sequenced on an Illumina MiSeq instrument generating 250-nt reads resulting in a mean per-base coverage of 75× (minimum 51× and maximum 108×).

Bioinformatics analysis

Raw reads were assessed using FastQC v0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), trimmed with Trimmomatic v0.36.6 (LEADING:10, TRAILING:10, SLIDINGWINDOW:4:20, and MINLEN:40) (44), assembled *de novo* with SPAdes v3.10.0 (45), and evaluated for assembly quality using Quast v5.0.2 (46). Bacterial identification was performed using PathogenWatch (https://pathogen.watch/), and ST assignment was determined using the PubMLST (https://pubmlst.org/) scheme. Bacterial genomes with novel alleles were submitted to BIGSdb (https://pubmlst.org/software/bigsdb) for analysis and ST assignment. Virulence factors of *E. coli* were screened using VirulenceFinder v2.0 (https://cge.food.dtu.dk/services/VirulenceFinder/), and virulence factors and predicted capsule (K) and lipopolysaccharide (O) profiles of *Klebsiella* species were determined using Kleborate v2.3.2. AMR genes were identified using

AMRFinderPlus (coverage length ≥ 90%, nucleotide identity ≥ 90%, without gaps) (47). *E. coli* phylogroups were determined *in silico* using the ClermonTyping algorithm (http://clermontyping.iame-research.center/), and O and flagellar (H) serotypes were determined using SeroTypeFinder v2 (https://cge.food.dtu.dk/services/SerotypeFinder/).

Genome comparisons

K. pneumoniae genomes belonging to ST147 were aligned to the reference genome NTUH-K2044, and recombinant regions were filtered out using Gubbins v3.3.0. Pairwise SNP differences between genomes were calculated and estimated using snp-dists (https://github.com/tseemann/snp-dists) (48). Contigs bearing the KPC-2 and NDM-1 genes were extracted from *de novo* assemblies and annotated with Prokka v1.5 (49). Since both genes were detected in transposable genetic elements, they were examined and annotated using MobileElementFinder v1.0.3 (https://pypi.org/project/MobileElementFinder/) and ISfinder (https://isfinder.biotoul.fr/) and then characterized using TETyper v1.1 (https://github.com/aesheppard/TETyper). Transposons were characterized using the Transposon Registry (http://transposon.lstmed.ac.uk/tn-registry) (50).

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DATA AVAILABILITY

The raw read files and assemblies for the five isolates are available at NCBI under BioProject accession number PRJNA865026.

ETHICS APPROVAL

The study protocol was approved by the Institutional Review Board of the Universidad Peruana Cayetano Heredia (SIDISI 100535) and approved by the Hospital Arzobispo Loayza research board. Patient information was anonymized and deidentified before analysis.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Table S1 (Spectrum02503-23-s0001.xlsx). MIC values for the studied isolates of carbapenemase-producing Enterobacterales (CPE).
Table S2 (Spectrum02503-23-s0002.xlsx). Virulence and antimicrobial resistance genes, along with assembly statistics of studied isolates of CPE.

Open Peer Review

PEER REVIEW HISTORY (review-history.pdf). An accounting of the reviewer comments and feedback.

REFERENCES

1. Tilahun M, Kassa Y, Gedefie A, Ashagire M. 2021. Emerging carbapenem-resistant *Enterobacteriaceae* infection, its epidemiology and novel treatment options: a review. Infect Drug Resist 14:4363–4374. https://doi.org/10.2147/IDR.S337611
2. Sheu C-C, Chang Y-T, Lin S-Y, Chen Y-H, Hsueh P-R. 2019. Infections caused by carbapenem-resistant *Enterobacteriaceae*: an update on therapeutic options. Front Microbiol 10:80. https://doi.org/10.3389/fmicb.2019.00080
3. Khuntayaporn P, Thirapanmethee K, Chomnawang MT. 2022. An update of mobile colistin resistance in non-fermentative gram-negative bacilli. Front Cell Infect Microbiol 12:882236. https://doi.org/10.3389/fcimb.2022.882236

4. GBD 2019 Antimicrobial Resistance Collaborators. 2022. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the global burden of disease study 2019. Lancet 400:2221–2248. https://doi.org/10.1016/S0140-6736(22)02185-7

5. Nordmann P, Naas T, Poirel L. 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 17:1791–1798. https://doi.org/10.3201/eid1710.110655

6. Beceiro A, Tomás M, Bou G. 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world Clin Microbiol Rev 26:185–230. https://doi.org/10.1128/CMR.00059-12

7. Amaretti A, Righini L, Candeliere F, Musmeci E, Bonvicini F, Gentilomi GA, Rossi M, Raimondi S. 2020. Antibiotic resistance, virulence factors, phenotyping, and genotyping of non-*Escherichia coli Enterobacterales* from the gut microbiota of healthy subjects. Int J Mol Sci 21:1847. https://doi.org/10.3390/ijms21051847

8. Velásquez J, Hernández R, Pamo O, Candiotti M, Pinedo Y, Sacsaquispe R, Cáceres L, Fernández N. 2013. *Klebsiella pneumoniae* resistente a los carbapenemes. primer caso de carbapenemasa tipo KPC en Perú. Rev Soc Peru Med Interna 26:192–196.

9. Horna G, Velasquez J, Fernández N, Tamariz J, Ruiz J. 2017. Characterisation of the first KPC-2-producing *Klebsiella pneumoniae* ST340 from Peru. J Glob Antimicrob Resist 9:36–40. https://doi.org/10.1016/j.jgar.2016.12.011

10. Mayta-Barrios MM, Ramirez-Illescas JJ, Pampa-Espinoza L, Yagui-Moscoso MJA. 2021. Molecular characterization of carbapenemases in Peru during 2019. Rev Peru Med Exp Salud Publica 38:113–118. https://doi.org/10.17843/rpmesp.2021.381.5882

11. Lezameta L, Cuicapuza D, Dávila-Barclay A, Torres S, Salvatierra G, Tsukayama P, Tamariz J. 2020. Draft genome sequence of a new Delhi metallo-β-lactamase (NDM-1)-producing *Providencia stuartii* strain isolated in Lima, Peru. Microbiol Resour Announc 9:e00788-20. https://doi.org/10.1128/MRA.00788-20

12. Roach D, Waalkes A, Abanto J, Zunt J, Cucho C, Soria J, Salipante SJ. 2020. Whole genome sequencing of Peruvian *Klebsiella pneumoniae* identifies novel plasmid vectors bearing carbapenem resistance gene *NDM-1*. Open Forum Infect Dis 7:faa266. https://doi.org/10.1093/ofid/ofaa266

13. Cuicapuza D, Alvarado L, Tocasca N, Aguilar D, Gómez-de-la-Torre JC, Salvatierra G, Tsukayama P, Tamariz J. 2023. First report of OXA-181-producing *Enterobacterales* isolates in Latin America. Microbiol Spectr 11:e0458422. https://doi.org/10.1128/spectrum.04584-22

14. Bevan ER, Jones AM, Hawkey PM. 2017. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145–2155. https://doi.org/10.1093/jac/dkx146

15. Gonzales E, Patiño L, Ore E, Martínez V, Moreno S, Cruzado NB, Rojas R, Quispe M del C, Carbonell I, Villarreal F, Maza G, Olivo J, Vicuña R, Bustamante D. 2020. β-lactamasas de espectro extendido tipo CTX-M en aislamientos clínicos de *Escherichia coli y Klebsiella pneumoniae* en el Instituto Nacional de Salud del Niño-Breña, Lima Perú. Rev Med Hered 30:242–248. https://doi.org/10.20453/rmh.v30i4.3659

16. Vásquez-Ponce F, Dantas K, Becerra J, Melocco G, Esposito F, Cardoso B, Rodrigues L, Lima K, de Lima AV, Sellera FP, Mattos R, Trevisoli L, Vianello MA, Sincero T, Di Conza J, Vespero E, Gutkind G, Sampaio J, Lincopan N. 2022. Detecting KPC-2 and NDM-1 coexpression in *Klebsiella pneumoniae* complex from human and animal hosts in South America. Microbiol Spectr 10:e0115922. https://doi.org/10.1128/spectrum.01159-22

17. Yang W, Hu F. 2022. Research updates of plasmid-mediated aminoglycoside resistance 16S rRNA methyltransferase. Antibiotics (Basel) 11:906. https://doi.org/10.3390/antibiotics11070906

18. Krause KM, Serio AW, Kane TR, Connolly LE. 2016. Aminoglycosides: an overview. Cold Spring Harb Perspect Med 6:a027029. https://doi.org/10.1101/cshperspect.a027029

19. Ruiz J. 2019. Transferable mechanisms of quinolone resistance from 1998 onward. Clin Microbiol Rev 32:e00007-19. https://doi.org/10.1128/CMR.00007-19

20. Ruiz J. 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J Antimicrob Chemother 51:1109–1117. https://doi.org/10.1093/jac/dkg222

21. Huang J, Li C, Song J, Velkov T, Wang L, Zhu Y, Li J. 2020. Regulating polymyxin resistance in gram-negative bacteria: roles of two-component systems PhoPQ and PmrAB. Future Microbiol 15:445–459. https://doi.org/10.2217/fmb-2019-0322

22. Luo Q, Yu W, Zhou K, Guo L, Shen P, Lu H, Huang C, Xu H, Xu S, Xiao Y, Li L. 2017. Molecular epidemiology and colistin resistant mechanism of *mcr*-positive and *mcr*-negative clinical isolated *Escherichia coli*. Front. Microbiol 8:2262. https://doi.org/10.3389/fmicb.2017.02262

23. Castanheira M, Costello AJ, Deshpande LM, Jones RN. 2012. Expansion of Clonal complex 258 KPC-2-producing Klebsiella pneumoniae in Latin American hospitals: Report of the SENTRY antimicrobial surveillance program. Antimicrob Agents Chemother 56:1668–1669. https://doi.org/10.1128/AAC.05942-11

24. Nascimento T, Cantamessa R, Melo L, Fernandes MR, Fraga E, Dropa M, Sato MIZ, Cerdeira L, Lincopan N. 2017. International high-risk clones of *Klebsiella pneumoniae* KPC-2/Cc258 and *Escherichia coli* CTX-M-15/CC10 in urban lake waters. Sci Total Environ 598:910–915. https://doi.org/10.1016/j.scitotenv.2017.03.207

25. David S, Reuter S, Harris SR, Glasner C, Feltwell T, Argimon S, Abudahab K, Goater R, Giani T, Errico G, et al. 2019. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. Nat Microbiol 4:1919–1929. https://doi.org/10.1038/s41564-019-0492-8

26. Pons MJ, Marí-Almirall M, Ymaña B, Moya-Salazar J, Muñoz L, Sauñe S, Salazar-Hernández R, Vila J, Roca I. 2020. Spread of ST348 *Klebsiella pneumoniae* producing NDM-1 in a Peruvian hospital. Microorganisms 8:1392. https://doi.org/10.3390/microorganisms8091392

27. Trigo da Roza F, Couto N, Carneiro C, Cunha E, Rosa T, Magalhães M, Tavares L, Novais Â, Peixe L, Rossen JW, Lamas LP, Oliveira M. 2019. Commonality of multidrug-resistant *Klebsiella pneumoniae* ST348 isolates in horses and humans in Portugal. Front Microbiol 10:1657. https://doi.org/10.3389/fmicb.2019.01657

28. Chen L, Chavda KD, Mediavilla JR, Jacobs MR, Levi MH, Bonomo RA, Kreiswirth BN. 2012. Partial excision of *bla*KPC from Tn4401 in carbapenem-resistant *Klebsiella pneumoniae*. Antimicrob Agents Chemother 56:1635–1638. https://doi.org/10.1128/AAC.06182-11

29. Cuzon G, Naas T, Nordmann P. 2011. Functional characterization of Tn4401, a Tn3-based transposon involved in *bla*KPC gene mobilization. Antimicrob Agents Chemother 55:5370–5373. https://doi.org/10.1128/AAC.05202-11

30. Piekar M, Álvarez VE, Knecht C, Leguina C, García Allende N, Carrera Páez L, Gambino AS, González Machuca A, Campos J, Fox B, Carpio E, Aguilar A, Alonso FM, Fernández Canigia L, Quiroga MP, Centrón D. 2023. Genomic data reveals the emergence of the co-occurrence of *bla*KPC-2 and *bla*CTX-M-15 in an *Escherichia coli* ST648 strain isolated from rectal SWAB within the framework of hospital surveillance. J Glob Antimicrob Resist 32:108–112. https://doi.org/10.1016/j.jgar.2022.12.012

31. Gonzales-Escalante E, Ruggiero M, Cerdeira L, Esposito F, Fontana H, Lincopan N, Gutkind G, Conza J. 2022. Whole-genome analysis of a high-risk clone of *Klebsiella pneumoniae* ST147 carrying both *mcr-1* and *bla*NDM-1 genes in Peru. Microb Drug Resist 28:171–179. https://doi.org/10.1089/mdr.2021.0128

32. Marquez-Ortiz RA, Haggerty L, Olarte N, Duarte C, Garza-Ramos U, Silva-Sanchez J, Castro BE, Sim EM, Beltran M, Moncada MV, Valderrama A, Castellanos JE, Charles IG, Vanegas N, Escobar-Perez J, Petty NK. 2017. Genomic epidemiology of NDM-1-encoding plasmids in Latin American clinical isolates reveals insights into the evolution of multidrug resistance. Genome Biol Evol 9:1725–1741. https://doi.org/10.1093/gbe/evx115

33. Lam MMC, Wyres KL, Judd LM, Wick RR, Jenney A, Brisse S, Holt KE. 2018. Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. Genome Med 10:77. https://doi.org/10.1186/s13073-018-0587-5

34. Wyres KL, Lam MMC, Holt KE. 2020. Population genomics of *Klebsiella pneumoniae*. Nat Rev Microbiol 18:344–359. https://doi.org/10.1038/s41579-019-0315-1

35. Wick RR, Heinz E, Holt KE, Wyres KL. 2018. Kaptive web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. J Clin Microbiol 56:e00197-18. https://doi.org/10.1128/JCM.00197-18

36. Brisse S, Passet V, Haugaard AB, Babosan A, Kassis-Chikhani N, Struve C, Decré D. 2013. Wzi gene sequencing, a rapid method for determination of capsular type for *Klebsiella* strains. J Clin Microbiol 51:4073–4078. https://doi.org/10.1128/JCM.01924-13

37. Garrity G, Brenner DJ, Krieg NR, Staley JR. 2005. The proteobacteria, part B: the gamma proteobacteria, p 1106. In Bergey's manual of systematic bacteriology. Springer, New York, NY.

38. Pasteran F, Gonzalez LJ, Albornoz E, Bahr G, Vila AJ, Corso A. 2016. Triton hodge test: improved protocol for modified hodge test for enhanced detection of NDM and other carbapenemase producers. J Clin Microbiol 54:640–649. https://doi.org/10.1128/JCM.01298-15

39. Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, Petropoulou D, Sofianou D. 2010. A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapene-mases in *Enterobacteriaceae* clinical isolates. J Antimicrob Chemother 65:1664–1671. https://doi.org/10.1093/jac/dkq210

40. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing. In CLSI supplement M100, 30th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

41. European Committee on Antimicrobial Susceptibility Testing. 2023. Clinical breakpoints and dosing of antibiotics. Available from: https://www.eucast.org/clinical_breakpoints. Retrieved 18 Apr 2023.

42. Simner PJ, Bergman Y, Trejo M, Roberts AA, Marayan R, Tekle T, Campeau S, Kazmi AQ, Bell DT, Lewis S, Tamma PD, Humphries R, Hindler JA. 2019. Two-site evaluation of the colistin broth disk elution test to determine colistin *in vitro* activity against gram-negative bacilli. J Clin Microbiol 57:e01163-18. https://doi.org/10.1128/JCM.01163-18

43. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18:268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

44. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

45. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021

46. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086

47. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W. 2021. Amrfinderplus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep 11:12728. https://doi.org/10.1038/s41598-021-91456-0

48. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153

49. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR. 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using gubbins. Nucleic Acids Res 43:e15. https://doi.org/10.1093/nar/gku1196

50. Roberts AP, Chandler M, Courvalin P, Guédon G, Mullany P, Pembroke T, Rood JI, Smith CJ, Summers AO, Tsuda M, Berg DE. 2008. Revised nomenclature for transposable genetic elements. Plasmid 60:167–173. https://doi.org/10.1016/j.plasmid.2008.08.001