Comparative genome analysis of colistin-resistant OXA-48-producing *Klebsiella pneumoniae* clinical strains isolated from two Iranian hospitals

Negin Bolourchi1, Fereshteh Shahcheraghi1, Christian G. Giske2, Shoeib Nematzadeh2, Narjes Noori Goodarzi3, Hamid Solgi4* and Farzad Badmasti1*

Abstract

**Background:** Carbapenemase-producing *Klebsiella pneumoniae* (CP-KP) is becoming extensively disseminated in Iranian medical centers. Colistin is among the few agents that retains its activity against CP-KP. However, the administration of colistin for treatment of carbapenem-resistant infections has increased resistance against this antibiotic. Therefore, the identification of genetic background of co-carbapenem, colistin-resistance *K. pneumoniae* (Co-CCRKp) is urgent for implementation of serious infection control strategies.

**Methods:** Fourteen Co-CCRKp strains obtained from routine microbiological examinations were subjected to molecular analysis of antimicrobial resistance (AMR) using whole genome sequencing (WGS).

**Results:** Nine of 14 *K. pneumoniae* strains belonged to sequence type (ST)-11 and 50% of the isolates had K-locus type 15. All strains carried *bla*<sub>OXA-48</sub> except for P26. *bla*<sub>NDM-1</sub> was detected in only two plasmids associated with P6 and P26 strains belonging to incompatibility (inc) groups; IncFIB, IncHI1B and IncFI. No *bla*<sub>KPC</sub>, *bla*<sub>IMP</sub> and *bla*<sub>IMP</sub> were identified. Multi-drug resistant (MDR) conjugative plasmids were identified in strains P6, P31, P35, P38 and P40. MIC<sub>colistin</sub> of *K. pneumoniae* strains ranged from 4 to 32 µg/ml. Modification of PmrA, PmrB, PhoQ, RamA and CrrB regulators as well as MgrB was identified as the mechanism of colistin resistance in our isolates. Single amino acid polymorphisms (SAPs) in PhoQ (D150G) and PmrB (R256G) were identified in all strains except for P35 and P38. CrrB was absent in P37 and modified in P7 (A200E). Insertion of IS<sub>Kpn72</sub> (P32), establishment of stop codon (Q30*) (P35 and P38), nucleotides deletion (P37), and amino acid substitution at position 28 were identified in MgrB (P33 and P42). None of the isolates were positive for plasmid-mediated colistin resistance (*mcr*) genes. P35 and P38 strains carried *iutA*, *iucD*, *iucC*, *iucB* and *iucA* genes and are considered as MDR-hypervirulent strains. P6, P7 and P43 had ICE<sub>Kp4</sub> variant and ICE<sub>Kp3</sub> was identified in 78% of the strains with specific carriage in ST11.

**Conclusion:** In our study, different genetic modifications in chromosomal coding regions of some regulator genes resulted in phenotypic resistance to colistin. However, the extra-chromosomal colistin resistance through *mcr* genes

*Correspondence: hamid.solgi@gmail.com; fbadmasti2008@gmail.com; f_badmasti@pasteur.ac.ir
1 Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran
4 Department of Laboratory Medicine, Amin Hospital, Isfahan University of Medical Sciences, Isfahan, Iran
Full list of author information is available at the end of the article

© The Author(s) 2021. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Introduction

Carbapenemase-producing *Klebsiella pneumoniae* (CP-KP) has been established as a major cause of healthcare-associated infection in many geographic areas, with high morbidity and mortality [1]. Infection with CP-KP is a serious clinical problem because it is difficult to treat using conventional antibiotics. Resistance to carbapenems can be mediated by various mechanisms including the production of carbapenemases, including *K. pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), and oxacillinase-48 (OXA-48), production of extended spectrum β-lactamases (ESBL) plus porins and hyperproduction of Ambler class C (AmpC) β-lactamase [2]. Among major carbapenemases, OXA-48 carbapenemase is currently the one that is spreading the most rapidly in the Middle East and other countries worldwide [3–5]. Polymyxins especially colistin are among the few agents that retain its activity against CP-KP, and they are considered as the key components against severe infections caused by these superbugs. Increasing administration of colistin for treatment of CP-KP infections has contributed to the emergence of acquired resistance against this antibiotic [6]. Resistance to colistin is mostly associated with LPS modification (result from mutations in *pmrA/pmrB*, *phoP/phoQ*, *mgrB*, *crrB*, and *ramA* genes) as well as overproduction of efflux-pumps (mediated by *kpnE/kpnF* and mutations of *acrB*) on the chromosomal level. Additionally, the production of phosphor-ethanolamine transferase encoded by plasmid-mediated colistin resistance (*mcr*) genes results in transferable colistin-resistance [7]. In Iran, where the carbapenemase is becoming extensively disseminated among clinical Gram-negative isolates, increasing rate of colistin-resistant *K. pneumoniae* is a critical matter. Therefore, the identification of genetic background of co-carbapenem, colistin-resistance *K. pneumoniae* (Co-CCRKP) is urgent for implementation of serious infection control strategies.

Methods

Bacterial isolates

In the period between January 2014 to March 2016, one hundred and thirty-eight carbapenem-resistant *K. pneumoniae* isolates were obtained from routine microbiological examinations on clinical samples (e.g., urine and bronchial aspirate) from two medical centers in two provinces of Iran (hospital A, a 496-bed university hospital, located in Tehran and hospital B, a 800-bed university hospital, located in Isfahan.). Conventional biochemical examinations were used for identification of the isolates. Genus and specie of isolates were confirmed by PCR-sequencing of 16s rRNA [10].

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using Kirby-Bauer disk diffusion method according to the clinical and laboratory standards institute (CLSI) guideline [11]. The minimal inhibitory concentration (MIC) of carbapenems (imipenem, meropenem and ertapenem) were determined by gradient test strips (Liofilchem, Italy). Broth microdilution method was utilized to determine the MIC of colistin using colistin sulfate (Merck, Germany). According to CLSI M100- S30, a MIC of ≥ 2 µg/ml was interpreted as intermediate susceptibility, whereas a MIC of ≥ 4 µg/ml was considered as resistance [11]. *E. coli* ATCC 25922 was used as a control strain for antimicrobial susceptibility testing.

DNA extraction and whole genome sequencing

DNA extraction was performed using a bacterial genomic DNA kit (GenElute™, Sigma). Genome libraries were obtained from Illumina System (Illumina Inc., San Diego, CA).
De novo assembly
The quality scores of the FASTQ paired-end files were checked using FastQC software [12]. Trimming and de novo assembly of short-read sequences was performed using Trimmomatic version 0.40 and SPAdes version 3.15.2, respectively [13] with k-mer = 99. The quality of contig assembly was assessed using QUAST software [14].

Post de novo assembly
Genomic statistics of contig assemblies including genome length, GC content, N50 and number of coding sequences (CDSs), rRNA and tRNA as well as gene annotation were determined using DFAST (https://dfast.ddbj.nig.ac.jp/). Chromosomal and extra-chromosomal contigs were sorted using mplasmids—version 1.0.0 (https://sarrendon.shinyapps.io/mlplasmids/). Multilocus sequence typing (MLST) of chromosomal contigs was determined using MLST Server 2.0 (https://cge.cbs.dtu.dk/services/MLST/). Plasmid incompatibility (Inc) was checked using PlasmidFinder 2.0 server (http://cge.cbs.dtu.dk/services/PlasmidFinder/). Antibiotic resistance genes were detected using the comprehensive antibiotic resistance database (CARD) (https://card.mcmaster.ca/analyze/rgi). Capsular typing (K-typing), O typing and allelic determination were performed using the Kaptive webtool (https://kaptive-web.erc.monash.edu/). Taxonomic and phylogenetic analysis was performed using Type Strain Genome Server (https://tygs.dsmz.de/). Modification of phylogenetic tree was performed using the iTOL webtool (https://itol.embl.de). The structures of interactive conjugative element of K. pneumoniae (ICEKp) were characterized using NCBI BLAST (https://www.ncbi.nlm.nih.gov). Single amino acid polymorphisms (SAPS) of chromosomally-encoded proteins involved in AMR were identified using paired-wise alignment in NCBI P-BLAST.

Results
K. pneumoniae isolates and antimicrobial susceptibilities
Totally, 14 of 138 carbapenem-resistant K. pneumoniae isolates (10.1%) (P6, P7, P26, P31, P32, P33, P35, P36, P37, P38, P40, P41, P42, P43 and P44) showed colistin-resistant phenotype. All 14 strains were resistant to cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), ciprofloxacin (CIP), ertapenem (ETP), meropenem (MEM) and imipenem (IMP). Strains were confirmed as colistin-resistant by broth microdilution method (according to 2020 CLSI M100-S30) with MICs ranged between 0.5 to 32 µg/ml. E-test results ranged from 0.5 to 32 µg/ml for ertapenem, 0.5 to 32 µg/ml for meropenem and 4 to 256 µg/ml for imipenem. Of 14 Co-CCRKp isolates, seven (50%) were isolated from intensive care unit (ICU), three from neurosurgery ward, two from emergency unit and two different isolates from a 55-year-old female outpatient. The strains were isolated from tracheal (7), urine (2), cerebrospinal fluid (2), blood (1), wound (1) and chest-tube (1). The clinical information of the strains is shown in Table 1.

Chromosomal characterization
Genetic features
The DFAST genetic analysis of purified contigs showed that chromosome length of 14 K. pneumoniae strains scaled from minimum 4,941,238 bp (for P38) to maximum 5,349,088 bp (for P37). The number of CDSs varied from 4694 to 5118. The highest N50 was calculated 215,119 belonging to P38. The GC content percentage for all isolated was 57%. The number of rRNA and tRNA varied from three to six and 51 to 80, respectively. See Additional file 1: Table S1.

Multilocus sequence typing (MLST)
The MLST results showed that eight strains (57%) belonged to ST11 (P31, P32, P33, P36, P40, P42, P43 and P44), three strains belonged to ST147 (P6, P7 and P26), two strains belonged to ST893 (P35, P38) and one strains belonged to ST101 (P37). Figure 1. See Additional file 1: Table S1.

K-locus and O typing
The results of K-locus typing showed that P6, P7 and P26 had K107D1 (wzc64 and wzi64); P33, P35 and P38 had K20 (wzc21 and wzi150); P37 had K17 (wzc18 and wzi150); P31, P32 and P39 had K10 D1-2 (wzc64 and wzi64); P40, P41, P42, P43 and P44 had K10 D1 (wzc64 and wzi64).

Table 1 Clinical information of 14 colistin-resistant OXA-48-producing K. pneumoniae strains

| Strain | Infection source | Hospital ward | Gender | Age | MIC Colistin |
|--------|------------------|---------------|--------|-----|--------------|
| P6     | Urine            | Outpatient    | Female | 55  | 16           |
| P7     | Wound            | Outpatient    | Female | 55  | 16           |
| P26    | Tracheal         | ICU           | Male   | 43  | 4            |
| P31    | Tracheal         | ICU           | Female | 22  | 16           |
| P32    | Tracheal         | ICU           | Female | 65  | 16           |
| P33    | Tracheal         | Neurosurgery  | Female | 34  | 16           |
| P35    | Urine            | ICU           | Male   | 77  | 16           |
| P36    | Chest tube       | ICU           | Male   | 26  | 16           |
| P37    | Tracheal         | ICU           | Male   | 37  | 16           |
| P38    | CSF              | ICU           | Female | 45  | 16           |
| P40    | Tracheal         | Neurosurgery  | Male   | 38  | 32           |
| P42    | Tracheal         | Neurosurgery  | Female | 34  | 32           |
| P43    | CSF              | Emergency     | Male   | 57  | 32           |
| P44    | Blood            | Emergency     | Female | 53  | 16           |
wzi 137) and the remained strains had K15 allelic profile (wzc 919 and wzi 50) (prevalence rate of 50%). Figure 1. In case of O-varient, P6, P7 and P26 belonged to O2v1 type; P35 and P38 belonged to O3/O3a; P37 belonged to O1v1 and P31, P32, P33, P36, P40, P42, P43 as well as P44 belonged to O4. See Fig. 1. See Additional file 1: Table S1.

AMR analysis

Resistance genes

The CARD results showed that our strains encoded AMR genes from different antibiotic classes including β-lactamases (blaSHV family and ampH1), ATP-binding cassette (ABC) antibiotic efflux pumps (lptD and msbA), major facilitator superfamily (mfs, h-NS, emrR, tetB and tetR), antibiotic efflux pumps (kpnE, kpnG, kpnH and kpnG), resistance-nodulation-cell division (RND) antibiotic efflux pumps (oqxA and oqxB, marA, adeF, baeR, rsmA and crp), pmr phosphoethanolamine transferase (epxB and arnT), bacterial porins (OmpK37 and OmpA) and fosfomycin thiol transferase (fosA6 and fosA5). See Additional file 1: Table S1.

Single amino acid polymorphisms (SAPs)

Single amino acid substitutions of chromosomally-encoded proteins involved in AMR were detected by comparing related coding regions of each strains with K. pneumoniae NTUH-K2044 (NC_012731) as the reference colistin-susceptible strain using paired-wise alignment. SAPs resulted in resistance to cephalosporins; PBP3, elfamycin; EF-Tu, fosfomycin; UhpT, fluoroquinolones; ParC, GyrA and GyrB and multiple drugs; MarR were identified. Table 2. All 14 strains underwent identical modifications in PBP3 (D350N, S357N), UhpT (E350Q) and EF-Tu (R234F). S3N (in P6, P7 and P26) and E86K (in P32, P33, P36, P37, P40, P42, P43 and P44) were detected for MarR protein. MarR was found intact in strains P31, P35 and P38. See Table 2. S80I was detected for ParC in all strains except for P38 (P403A). GyrA was modified identically in all strains (S83I) except for P37 and P43 (D87G). GyrB was found intact in all strains except for P6, P7, P26 and P43 (E466D). Regarding colistin-resistance; mgrB was found intact in all strains except for P37 and P38 (E466D). mgrB was found intact in P6, P7, P26, P31, P36, P37, P40, P42, P43 and P44. However, P35 and P38 underwent a stop codon (Q30*). Alteration of MgrB in P32 was mediated by insertion of ISKpn72. Moreover, P37 had a large deletion and frame shift in the mgrB coding region. Amino acid modification (C28W) of MgrB was detected in P33 and P42. SAP of PhoQ (D150G) was identified in all strains except for P35 and P38. No amino acid alteration was found related to PhoP and AcrB. Amino acid substitutions of PmrA were detected in P35 and P42. SAP in PmrB (R256G) was identified in all strains except for P35 and P38. No amino acid alteration was found related to PmrB. Amino acid modifications in P6, P7, P26 (D71B) and P43 (I25T). See Table 2. Modification of CrrB was only detected in P7 (A200E). Interestingly, CrrB was absent in P37.
| Protein | Strains          | Cephalosporin resistance | Elfamycin resistance | Fosfomycin resistance | Repressor of multi-drug resistance operon | Fluoroquinolone resistance | Colistin resistance |
|---------|------------------|--------------------------|----------------------|-----------------------|------------------------------------------|-----------------------------|---------------------|
|         | PBP3             | EF-Tu                    | UhpT                 | MarR                  | ParC                                     | GyrA                        | GyrB                | PhoP | PhoQ | PmrA | PmrB | MgrB | RamA | AcrB | CrrB |
| P6      | D350N, S357N     | R234F                    | E350Q                | S3N                   | S80I                                     | S83I                        | E466D               | Intact | D150G | Intact | R256G | Intact | D71B | Intact | Intact |
| P7      | D350N, S357N     | R234F                    | E350Q                | S3N                   | S80I                                     | S83I                        | E466D               | Intact | D150G | Intact | R256G | Intact | D71B | Intact | Intact |
| P26     | D350N, S357N     | R234F                    | E350Q                | S3N                   | S80I                                     | S83I                        | E466D               | Intact | D150G | Intact | R256G | Intact | D71B | Intact | Intact |
| P31     | D350N, S357N     | R234F                    | E350Q                | Intact                | S80I                                     | S83I                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P32     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | S83I                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P33     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | S83I                        | Intact               | Intact | D150G | Intact | R256G | Intact | C28W  | Stop codon (Q30*) | Intact | Intact | Intact | Intact | Intact | Intact | Intact | Intact |
| P35     | D350N, S357N     | R234F                    | E350Q                | Intact                | S80I                                     | S83F                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P36     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | S83I                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P37     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | D87N                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P38     | D350N, S357N     | R234F                    | E350Q                | Intact                | P403A                                    | S83F                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P40     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | S83I                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P42     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | S83I                        | Intact               | Intact | D150G | Intact | R256G | Intact | C28W  | Intact | Intact |
| P43     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | D87G                        | E466D               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |
| P44     | D350N, S357N     | R234F                    | E350Q                | E86K                  | S80I                                     | S83I                        | Intact               | Intact | D150G | Intact | R256G | Intact | Intact | Intact | Intact |

*ND means not detected*
**Phylogenetic analysis**

The genome-based phylogenetic tree showed that our 14 Co-CCRKp strains are divided into four clades as follows: P35 and P38 = clade 1; P6, P7 and P26 = clade 2; P37 = clade 3 and P31, P32, P33, P36, P40, P42, P43 and P44 = clade 4. The dendrogram demonstrated that the phylogenetic categorization of the strains almost matched with their classification based on ST, K-type and O-type. See Fig. 1.

**Integrative conjugative element (ICEKp)**

Two distinct modules were identified in ICEKp structures of our strains: Yersiniabactin (ybtS, ybtX, ybtQ, ybtP, ybtA, irp2, irp1, ybtU, ybtT, ybtE, and fyuA) and self-mobilization (int, xis, virB1, virB2, virB4, virB5, virB6, virB8, virB9, virB10, virB11, mobB genes and oriT site). No colibactin was identified. ICEKp3 was the predominant ICEKp variant among 13 positive strains except for P23. P6, P7 and P43 carried ICEKp4 and the rest of the strains (78%) harbored ICEKp3. The virB3 and virB7 genes were absent in all ICEKp structures. The locus of virB was not identified in ICEKp of P7. See Fig. 2.

**Extra-chromosomal characterization**

**Genetic features**

Thirty-five putative plasmids were assembled. Strains P6, P35, P37, P38, P40 and P44 carried three plasmids and P7, P26, P31, P32, P33, P36, P42 and P43 carried two plasmids. Plasmids incompatibility (Inc) groups were identified as IncFIB = 16, IncFI1 = 12, IncHI1B = 6, ColI4401 = 4, IncL = 4 and repB = 2. See Fig. 3. One/two plasmids in P6, P7, P35, P38, P40 and P44 strains were positive for all four compartment of relaxasome complex including oriT, relaxase, type IV secretion system (T4SS) and type IV coupling protein (T4CP). P31, P32, P36 and P42 carried one plasmid lacking only T4CP. Integron class 1 was
detected in plasmids of all 14 K. pneumoniae strains. See Additional file 2: Table S2.

**AMR and hypervirulence analysis**

AMR genes related to multiple antibiotic drug classes were identified in majority (85%) of plasmids. ESBLs including blaOXA-48, blaCTX-M-15, blaTEM-1, and blaNDM-1 were identified. Figure 1. blaOXA-48 was detected in all Co-CCRKp isolates, with the exception of P26. Strains P6, P7, P35 and P38 encoded blaOXA-1, blaCTX-M-15 and blaTEM-1 were identified in 78% and 57% of the strains, respectively. The blaNDM-1 gene was found in only two plasmids related to strains P6 and P26 (both belonging to ST147). No blaKPC, blaVIM and blaIMP were identified. AMR genes related to aminoglycoside modifying enzymes including aph(3’)-lb, aac(6’)-lb-b9, aph(6’)-ld, aac(6’)-lb-cr16 adaA5, rmtC and rmtF were found in all strains except for P37 and P43. qnrS1 was detected in 71% of the strains. qnrB1 was identified only in plasmids related to P35 and P38. See Fig. 1. Other AMR genes were tetB, tetR, ermB, arr2, mphA, catB3 and dfrA14. No mcr was detected. Strains P35 and P38 carried iutA, iucD, iucC, iucB and iucA hypervirulence genes on their plasmids. See Additional file 2: Table S2.

**The blaOXA-48 and blaNDM-1 genetic environment**

The gene annotation of OXA-48-producing plasmids showed that blaOXA-48 was flanked upstream/downstream by the LysR family transcriptional factor (lysR) in almost all strains. Additional file 3: Figure S1A. In P31, blaOXA-48 was flanked downstream by insertion sequence (IS) element transposase and blaCTX-M-15. In P32, two blaOXA-48 genes were identified on two distinct regions which environment for one of them was similar to P31. The latter was flanked upstream by IS6 family transposase. In P33, the lysR and blaOXA-48 complex was flanked downstream by three hypothetical genes and a transposase. The lysR and blaOXA-48 complex, dhps, aph(6’)-ld and blaCTX-M-15 were surrounded by IS4 and a transposase element in P35. In P36, P37, P38 and P44, the lysR and blaOXA-48 complex was flanked upstream by IS911, IS4 and IS110 family transposase genes, respectively. No transposase element was found surrounding the lysR and blaOXA-48 complex in P6, P7, P40, P42 and P43. blaNDM-1 was flanked upstream by bleomycin binding protein and trpF genes and downstream by IS911 family transposase in both P6 and P26. See Additional file 3: Figure S1B.

**Discussion**

Our understanding about the genetic background of K. pneumoniae has increased worldwide. However, few WGS-based studies have focused on clinical K. pneumoniae isolates in Iran and consequently; the detailed genomic knowledge of circulating STs in clinical settings is still limited in our country. WGS data for 14 clinical Co-CCRKp strains isolated between 2014 and 2016 in Iran were analyzed to provide a comparative genetic background that will help us to upgrade our information regarding epidemiology of AMR determinants.

One of the most clinically important AMR genes in K. pneumoniae isolates is class D β-lactamase blaOXA-48 [15]. The blaOXA-48 gene has been carrying by various plasmids Inc types including IncL/M, IncN, and IncA/C [16]. However, the results of our plasmid replicon typing shows that blaOXA-48 is carried by various incompatibility groups including IncL, IncFII, IncFIB and mainly IncFIL. The genetic analysis of blaOXA-48 environment in our strains indicates that the LysR family transcriptional factor gene is closely related to blaOXA-48 carriage in K. pneumoniae. Detection of blaOXA-48 in transferrable plasmids is a clinical emergency as it can rapidly be widespread among other strains and even other Enterobacteriaceae mediating highly resistant outbreaks in healthcare settings. P6, P31, P35, P38 and P40 are considered clinically significant strains as they contain conjutative plasmids carrying blaOXA-48 carbapenemase. This suggests that carbapenem-resistance soon will disseminate in ICU and other wards among Iranian medical settings. Also, the carriage of a conjugative OXA-48-producing plasmids in an outpatient is highly troublesome as it implies the higher prevalence rates of carbapenemase in the community in near future. In the presented study, we reported the first extra-chromosomal carriage of two blaOXA-48 in strain P32. The double carriage of blaOXA-48 family by a single strain is rarely reported and is a matter of concern due to more rapid transmission of this
significant carbapenemase. Sherchan and colleagues reported two copies of \( bla_{OXA-48} \) on ST147 \( K. pneumoniae \) from Nepal in 2020 which was previously reported from Pakistan, the United Arab Emirates and Korea [17].

P6 and P26 strains were detected as the only NDM-1 producing strains in our study indicating lower rate of \( bla_{NDM-1} \) carriage compared to other β-lactamase such as \( bla_{CTX-M-15} \) and \( bla_{TEM-1} \). However, higher prevalence of \( bla_{NDM-1} \) was reported form other Middle Eastern countries specifically Egypt and Saudi Arabia [18, 19]. In the study by Ghaiht et al. [20] 52.2% of \( K. pneumoniae \) strains isolated from neonatal ICU in Cairo were NDM-1 producers. The prevalence of \( bla_{NDM-1} \) among \( Enterobacteriales \) is mediated by rapid dissemination of conjugative plasmids [16]. In our study, none of the NDM-1 producing plasmids were conjugative. \( bla_{NDM-1} \) gene has been detected on plasmids of various incompatibility groups including IncF, IncA/C, IncL/M, IncH, IncN, and IncX3 or untypeable [16]. However, both of NDM-1 producing plasmids in our study belonged to IncFIB and IncH1B. Co-carriage of \( bla_{NDM-1} \) and \( bla_{OXA-48} \) genes was seen in strain P6. Solgi et al. investigated the ESBLs carriage of 71 clinical carbapenem-resistant \( Enterobacteriales \) in an Iranian hospital in Tehran. In this study, among 62 bacterial isolates, 46% and 37% of the isolates harbored \( bla_{NDM-1} \) and \( bla_{OXA-48} \) respectively and co-carriage of \( bla_{NDM-1} \) and \( bla_{OXA-48} \) was detected in 16% of the isolates. Also, the plasmid incompatibility types IncFII and IncA/C were identified among the NDM-1 producing isolates, while only IncL/M was detected among OXA-48 producers [16]. Co-existence of the \( bla_{NDM-1} \) and \( bla_{OXA-48} \) genes among CR-KP isolates was previously reported from other Middle Eastern countries such as Egypt. In a recent study by El-Domany and colleagues [21], 50 isolates co-carrying \( bla_{NDM-1} \) and \( bla_{OXA-48} \) were reported among 230 \( K. pneumoniae \) clinical isolates. In this study, the rate of \( bla_{NDM-1} \) and \( bla_{OXA-48} \) was reported 70.0% and 52.0%, respectively. In our study, ST11, ST147, ST1993 and ST101 were \( bla_{OXA-48} \) and \( bla_{NDM-1} \) carriers in agreement with previous reports of OXA-48 and NDM-1 producing \( K. pneumoniae \) from Iran [16, 22]. These consensus reports suggest successful circulation of mentioned STs in Iran. The clonal carriage of \( bla_{OXA-48} \) and \( bla_{NDM-1} \) among \( K. pneumoniae \) strains may be region-specific in a single geographical territory despite high burden of individual transits. Accordingly, a significant association was reported for ST199 and ST152 with \( bla_{OXA-48} \) and \( bla_{NDM-1} \) carriage form Saudi Arabia, respectively [23, 24].

Co-existence of \( bla_{OXA-48} \), \( bla_{CTX-M-15} \) and \( bla_{TEM-1} \) within one single plasmid was observed in P6, P31, P32, P35, P38 and P44. However, this complex production was also observed in separate plasmids of P7, P40 and P42. Production of OXA-48 in \( K. pneumoniae \) plasmids seems to be typically concurrent with other ESBLs mainly \( bla_{CTX-M} \) and \( bla_{TEM} \). In one study, 88 of 94 of \( K. pneumoniae \) isolates in an Iranian hospital harbored \( bla_{SHV} \), \( bla_{CTX-M-15} \) and \( bla_{TEM-1} \) concurrently while only one and two isolates solely carried \( bla_{CTX-M-15} \) and \( bla_{SHV} \) respectively [15]. The transmission of major ESBLs among \( K. pneumoniae \) has been reported in conjugative IncI/M and IncFII plasmids with ability of interspecies transfer [23, 25]. Accordingly, in our extra-chromosomal analysis, IncFII was a major plasmid replicon type and IncL was detected in two conjugative ESBL producer plasmids.

Resistance to colistin has been increasingly reported in the world including the Middle East region. A high resistance rate of 16.9% was reported between 2015 and 2016 from Iran [26]. Totally, 524 colistin-resistant \( K. pneumoniae \) isolates were reported from Turkey and Iran between 2013 and 2018 [27]. Jafari et al. [28] reported an increase of colistin resistance up to 50% in carbapenem-resistant \( K. pneumoniae \) isolates.

In the presented study; amino acid substitution, premature termination, deletion and insertion of IS element were identified in the coding region of MgrB as well as PmrA/PmrB, PhoP/PhoQ, RamA and CrrB regulators. MgrB inactivation was identified in 42% of the strains including P32, P33, P35, P37, P38 and P42. Substitution of cysteine at position 28 of MgrB with tryptophan was detected in P33 and P42. Position 28 is considered as an important region for amino acid substitution due to the key role of disulfide bonds of cysteine residue in MgrB functionality [29]. Similar SAPs such as C28F, C28Y and C28S has been previously reported in several different studies [6, 30]. In addition, truncation of MgrB at position 30 (Q30*) was identified in P35 and P38. Position 30 is highly prone to be modified in long exposure to colistin and therefore; (Q30*) has been commonly reported in other studies [29]. Insertional inactivation of MgrB was identified in only one strain (P32) mediating by insertion of IS\text{Kpn}72 element (belonging to IS\text{4} family) in coding region of MgrB. Zhang et al. recently demonstrated the phenotypic switch of colistin-susceptible \( K. pneumoniae \) strains to colistin-resistant ones by horizontal transfer of an IS\text{Kpn}72 carrying-plasmid. Therefore, in spite of chromosomal origin of MgrB; its modification can be plasmid-mediated and depended on conjugation processes. However, this phenomenon is not probable in our study because both plasmids of P32 were non-conjugative [31]. In this study, \textit{crr}B gene was not carried by the P37 chromosome. The absence of \textit{crr}B was previously reported in the study by Jayol et al. [32] which may be due to the differences in the lateral acquisition of the \textit{crr}AB operon in \( K. pneumoniae \). Thomas et al. [33] claimed that the lack of \textit{Crr}B leads to significantly higher MIC\textsubscript{colistin} and bacterial
virulence than MgrB disruption in CR-KP. However, it seems that such manifestation cannot be attributed to P37. SAPs in PhoQ and PmrB were detected in 85% and 78% of the strains, respectively. PhoQ and PmrB modifications have been frequently reported in colistin-resistant \textit{K. pneumoniae} isolates. In the study by Haelli et al. \cite{26} 95% of 20 \textit{K. pneumoniae} strains underwent point mutations in PmrB of which R256G was detected in five strains. However, the amino acid substitution in PhoQ or PmrB does not always correspond colistin-resistance \cite{30, 34, 35}. Zhu et al. \cite{36} reported mutated PhoQ in seven colistin-susceptible isolates of \textit{K. pneumoniae}. The authors also suggested that the exposure of the isolates to colistin during treatment resulted in new PhoQ alterations mediating resistance to colistin. It is evident that upregulation of \textit{pmrHFIIJKLM}, \textit{pmrCAB}, as well as \textit{pmrK} operons is associated with colistin resistance in \textit{K. pneumoniae} \cite{37, 38}. Therefore, the mechanism of colistin resistance needs to be carefully interpreted.

The prevalence of hypervirulence among MDR \textit{K. pneumoniae} is increasing worldwide \cite{39}. In a meta-analysis conducted by Sanikhani et al. \cite{40} the rate of reported hypervirulent \textit{K. pneumoniae} isolates was determined 21.7% globally which majority of them were from China. In our study, P35 and P38 carried non-conjugal hypervirulence plasmids belonging to repB plasmid replicon type and considered as MDR-hypervirulent strains. This may suggest that P35 and P38 possess hypervirulent background which acquired MDR plasmids through conjugal processes. The rate of hypervirulence was 14% in our study and both strains were positive for \textit{iutA}, \textit{iucD}, \textit{iucC}, \textit{iucB} and \textit{iucA}. Few studies have reported hypervirulent \textit{K. pneumoniae} in Iran. Pajand et al. investigated the presence of hypervirulence genes in \textit{K. pneumoniae} clinical isolates. \textit{peg344}, \textit{iucA}, \textit{rmpA}, \textit{rmpA2}, \textit{iroB1}, \textit{iroB2} and \textit{iutA} were detected among all carbapenem-resistant isolates with 1.8%, 1.8%, 1.8%, 1.8%, 7.3%, 12.7% and 18.2% rate of carriage, respectively. The results of their study indicated that the prevalence of \textit{iutA} was even higher in NDM-1 producing isolates \cite{41}. Taraghian et al. detected 11 MDR-hypervirulent strains among 105 urinary tract \textit{K. pneumoniae} isolates. In their study, ESBLs were identified in all hypervirulent strains and their carriage by hypervirulent strains were significantly higher compared to the classical ones \cite{42}. In addition, Sanikhani et al. \cite{43} reported the prevalence rate of 21.38% among 477 \textit{K. pneumoniae} clinical isolates with high percentage of MDR and high-level resistance to imipenem.

The ICEKp analysis highlighted that the majority of our strains (78%) carried ICEKp3. According to an investigation by Lam et al. among 2498 \textit{K. pneumoniae} genomes belonging to 37 different STs; ICEKp4, ICEKp3, ICEKp10 and ICEKp5 were the most common types of ICEKp. The results of this study also revealed that ICEKp variants can be integrated more frequently in specific STs compared to the other ones. The authors showed that almost 40% of the ICEKp positive \textit{K. pneumoniae} strains belonged to CC258 \cite{44}. Similarly, another study from South America indicated that the rate of ICEKp carriage is higher in strains belonging to ST11 and ST340. In our study, almost all ST11 strains harbored ICEKp3. This specific carriage of ICEKp3 in \textit{K. pneumoniae} ST11 was previously reported by a Turkish study in 2019 \cite{45}.

**Conclusion**

In this study, we assembled the whole-genomes of 14 colistin-resistant OXA-48-producing \textit{K. pneumoniae} referred to two Iranian hospitals as well as their plasmids. The prevalence of \textit{bla\textsubscript{OXA-48}} in our strains was very high as well as \textit{bla\textsubscript{SHV}}, \textit{bla\textsubscript{CTX-M-15}} and \textit{bla\textsubscript{TEM-1}}. MDR conjugative plasmids as well as hypervirulent plasmids were detected in some strains. Amino acid substitution, premature termination, deletion and insertion of an IS element were identified in the coding region of MgrB. SAPs in PmrA, PmrB, PhoQ, RamA and CrrB regulators were involved in colistin resistance. Pan-genomic investigations can provide data on AMR reservoir of highly-resistant \textit{K. pneumoniae} at clinical and even community levels and ultimately provide a realistic status of AMR countrywide.

**Abbreviations**

AMR: Antimicrobial resistance; WGS: Whole genome sequencing; MDR: Multi-drug resistant; SAPs: Single amino acid polymorphisms; mcr: Plasmid-mediated colistin resistance; Co-CCRKp: Co-carbenem colistin-resistant \textit{K. pneumoniae}; CP-KP: Carbapenemase-producing \textit{K. pneumoniae}; KPC: \textit{K. pneumoniae} carbapenemase; CLSI: Clinical and laboratory standards institute; MIC: Minimal inhibitory concentration; CDSs: Coding sequences; CARD: Comprehensive antibiotic resistance database; ICEKp: Interactive conjugative element of \textit{K. pneumoniae}; ABC: ATP-binding cassette; RND: Resistance-nodulation-cell division; NDM: New Delhi metallo-\textit{β}-lactamase; ESBL: Extended spectrum \textit{β}-lactamases; AmpC: Ambler class C; T4SS: type IV secretion system; TACP: Type IV coupling protein; ICU: Intensive care unit; IS: Insertion sequence; Inc: Incompatibility.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12941-021-00479-y.

**Additional file 1:** Table S1. Genotypic, resistome profile and capsular and O-typing of 14 colistin-resistant OXA-48-producing \textit{K. pneumoniae} strains.

**Additional file 2:** Table S2. Extrachromosomal Genetic and resistome information of 14 colistin-resistant OXA-48-producing \textit{K. pneumoniae} strains.

**Additional file 3:** Figure S1. A) The genetic environment of \textit{bla\textsubscript{OXA-48}} and \textit{bla\textsubscript{KPC}}. B) The genetic environment of \textit{bla\textsubscript{OXA-48}} in 14-carbon-resistant OXA-48-producing \textit{K. pneumoniae} strains. \textit{bla\textsubscript{OXA-48}} was identified in all strains except for P26. The \textit{fysR} and \textit{fysR} complex was found in all strains. No transposase element was detected surrounding the \textit{fysR} and \textit{fysR} complex in P6, P7, P40, P42 and P43. Two \textit{bla\textsubscript{OXA-48}} with different genetic arrangement were found on
P32 plasmid. In P36, P37, P38 and P44, the lexR and blaOXA-48 Complex was flanked upstream by IS1216, IS1243 and IS1101 family transposase genes, respectively. The blaOXA-48 was detected only in P6 and P26. blaOXA-48 was flanked upstream by blomycin binding protein and trpF genes and downstream by the IS59 family transposase.

Acknowledgements

The authors would like to thank the personnel in the bacteriology department of Pasteur Institute of Iran for their help. This research was supported by Pasteur Institute of Iran.

Authors’ contributions

FB, HS, CGG and NB designed the study and drafted the manuscript. FB, HS, CGG, SN, NNG and NB performed the experimental work and analyzed the data. FB read and approved the final manuscript. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant or funding.

Availability of data and materials

All generated data and materials are included in the text. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the BioProject accession number PRJNA499021. The genomes described in this paper are version JAHYXPC000000000.JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000, JAHYXPC000000000.

Declarations

Ethics approval and consent to participate

This project was conducted based on the ethical guidelines previously approved by the Pasteur Institute of Iran (project number: IR.PII.REC.0.1395.51).

Consent for publication

Authors have read the manuscript and consented for publication of all presented information.

Competing interests

Authors declare that they had no commercial, personal and political conflicting of interests.

Author details

1. Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran. 2. Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden. 3. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. 4. Department of Laboratory Medicine, Amin Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.

Received: 23 August 2021 Accepted: 7 October 2021

Published online: 23 October 2021

References

1. Spynidopoulou K, Psichogiou M, Sypsa V, Miriagou V, Karapanou A, Hadjihannas L, Tsouvelakis L, Daikos GL. Containing Carbapenemase-producing Klebsiella pneumoniae in an endemic setting. Antimicrob Resist Infect Control. 2020;9(1):102.
2. Codjoe FS, Donkor ES. Carbapenem resistance: a review. Med Sci (Basel, Switzerland). 2017;6(1):1.
3. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, Zheng Y, Guo Y, Zhang R, Hu F. Dissemination of carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant Enterobacteriaceae isolated from adult and children patients in China. Front Cell Infect Microbiol. 2020;10:314.
4. Mairi A, Pantel A, Sotto A, Lavigne JP, Touati A. OXA-48-like carbapenemases producing Enterobacteriaceae in different niches. Eur J Clin Microbiol Infect Dis. 2018;37(6):587–604.
5. Pitout JDD, Peirano G, Lock MM, Strydom K-A, Matsumura Y. The global dissemination of OXA-48-type carbapenemases. Clin Microbiol Rev. 2019;33(1):e00102-19.
6. Palmieri M, D’Andrea MM, Pelegrin AC, Mirande C, Brkic S, Cirkovic I, Goossens H, Rossolini GM, van Belkum A. Genomic epidemiology of carbapenem- and colistin-resistant Klebsiella pneumoniae isolates from serbia: predominance of ST101 strains carrying a novel OXA-48 plasmid. Front Microbiol. 2020;11:294.
7. Aghapour Z, Gholidadeh P, Ganbarov K, Bialvaei AZ, Mahmood SS, Tanomand A, Yousefi M, Asgharzadeh M, Yousefi B, Kafil HS. Molecular mechanisms related to colistin resistance in Enterobacteriaceae. Infect Drug Resist. 2019;12:965–75.
8. Bowers JR, Kitchell B, Driebe EM, MacCannell DR, Roe C, Lemmer D, de Man T, Rasheed JK, Engelthaler DM, Keim P, et al. Genomic analysis of the emergence and rapid global dissemination of the clonal group 258 Klebsiella pneumoniae pandemic. PLoS ONE. 2015;10(7):e0133722.
9. Musicha F, Mufelia CL, Mather AE, Chaguzza C, Cain AK, Peno C, Kallonen T, Khonga M, Denis B, Gray KJ, et al. Genomic analysis of Klebsiella pneumoniae isolates from Malawi reveals acquisition of multiple ESBL determinants across diverse lineages. J Antimicrob Chemother. 2019;74(5):1233–22.
10. Juettechko S, Loy A, Lehner A, Wagner M. The microbial community composition of a nitrifying-denitrifying activated sludge from an industrial sewage treatment plant analyzed by the full-cycle rRNA approach. Syst Appl Microbiol. 2002;25(1):84–99.
11. CLSI. Performance standards for antimicrobial susceptibility testing. In: Edited by Institute CLS, 30th edn. 2020.
12. Brown J, Purrung M, McCue LA. FQC Dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. Bioinform. 2017;33(19):3137–9.
13. Bankевич A, Nukl S, Antipov D, Guryevich AA, Dvoirin M, Kulikov AS, Lein VM, Nikolенко S, Pham S, Pyribetski AD. SPades: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455–77.
14. Guryevich A, Saveliev V, Vyalhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinform. 2013;29(8):1072–5.
15. Solgi H, Badmasti F, Giske CG, Aghamohammadi S, Shahcheraghi F. Molecular epidemiology of NDM-1-producing Klebsiella pneumoniae in an Iranian hospital: clonal dissemination of ST11 and ST893. J antimicrob Chemother. 2018;73(6):1517–24.
16. Solgi H, Nematzadeh S, Giske CG, Badmasti F, Westerlund F, Lin YL, Goyal G, Nikbin VS, Nemat AH, Shahcheraghi F. Molecular epidemiology of OXA-48 and NDM-1 producing Enterobacteriaceales species at a University Hospital in Tehran, Iran. Between 2015 and 2016. Front Microbiol. 2020;11:936.
17. Shcherban JB, Tada T, Sheetha S, Uchida H, Hishinuma T, Morioka S, Shahi K, Bhandari S, Twi RT, Kirikae T, et al. Emergence of clinical isolates of highly carbapenem-resistant Klebsiella pneumoniae co-harboring blaNDM-1 and blaOXA-48 in Nepal: Int J Infect Dis. 2020;92:247–52.
18. Khalil MA, Elgamal A, El-Mowafy M. Emergence of multdrug-resistant New Delhi metallo-β-lactamase-1-producing Klebsiella pneumoniae in Egypt. Microb Drug Resist. 2017;23(4):480–7.
19. Touati A, Mairi A. Epidemiology of carbapenemase-producing Enterobacteriaceae in the Middle East: a systematic review. Expert Rev Anti Infect Ther. 2020;18(3):241–50.
20. Ghahati DM, Ziaei MW, Said HM, Elnawany S, Eltaban S, Al-agamy MH, Bohol MFE, Bendary MM, Al-Qahatani A, Al-Ahdal MN. Genetic diversity of carbapenem-resistant Klebsiella pneumoniae causing neonatal sepsis in intensive care unit, Cairo, Egypt. Eur J Clin Microbiol Infect Dis. 2020;39(3):583–91.
21. El-Domany RA, El-Banna T, Sonbol F, Abu-Sayedahmed SH. Co-existence of NDM-1 and OXA-48 genes in carbapenem resistant Klebsiella pneumoniae clinical isolates in Kafrelsheikh, Egypt. Afr Health Sci. 2021;21(2):489–96.
22. Solgi H, Shahcheraghi F, Bolourchi N, Ahmadi A. Molecular characterization of carbapenem-resistant serotype K1 hypervirulent Klebsiella pneumoniae ST11 harbouring blaNDM-1 and blaOXA-48 carbapenemases in Iran. Microb Pathog. 2020;149:104507.

23. Zaman TU, Alfordayan M, AlBadri M, Aldrees M, Siddique MI, Ajlouni S, Balkhy HH. Clonal diversity and genetic profiling of antibiotic resistance among multidrug/carbapenem-resistant Klebsiella pneumoniae isolates from a tertiary care hospital in Saudi Arabia. BMC Infect Dis. 2018;18(1):205.

24. Sonnevend Á, Ghazawi AA, Hashmey R, Jamal W, Rotimi VO, Shibl AM, Al-Adsari MA, Absi A. Molecular characterizations of carbapenem-resistant Enterobacteriaceae with high rate of autochthonous transmission in the Arabian Peninsula. PLoS ONE. 2015;10(6):e0131732.

25. Torres-González P, Bobadilla-Del Valle M, Tovar-Calderón E, Leal-Vega F, Hernández-Cruz A, Martínez-Gamboa A, Niembro-Ortega MD, Sifuentes-Osorio J, Porce-de-León A. Outbreak caused by Enterobacteriaceae harboring NDM-1 metallo-β-lactamase carried in an IncFII plasmid in a tertiary care hospital in Mexico City. Antimicrob Agents Chemother. 2015;59(11):7080–3.

26. Haeri M, Javani A, Moradi J, Jafari Z, Feizabadi MM, Babaei E. MgrB alterations mediate colistin resistance in Klebsiella pneumoniae isolates from Iran. Front Microbiol. 2017;8:2470.

27. Aris P, Robatjazi S, Nikkhahi F, Amin Marashi SM. Molecular mechanisms and prevalence of colistin resistance of Klebsiella pneumoniae in the Middle East region: a review over the last 5 years. J Glob Antimicrob Resist. 2020;22:625–30.

28. Jafari Z, Harati AA, Haeri M, Kardani-Yamchi J, Jafari S, Jabalameili F, Mey samie A, Abdollahi A, Feizabadi MM. Molecular epidemiology and drug resistance pattern of carbapenem-resistant Klebsiella pneumoniae isolates from Iran. Microb Drug Resist. 2019;25(3):336–43.

29. Kong Y, Li C, Chen H, Zheng W, Sun Q, Xie X, Zhang J, Ruan Z. In vivo emergence of colistin resistance in carbapenem-resistant Klebsiella pneumoniae mediated by premature termination of the mgrB Gene regulator. Front Microbiol. 2021;12:656610.

30. Cheng Y-H, Lin T-L, Pan Y-J, Wang Y-P, Lin Y-T, Wang J-T. Colistin resistance mechanisms in Klebsiella pneumoniae strains from Taiwan. Antimicrob Agents Chemother. 2015;59(5):2909–13.

31. Zhang B, Yu B, Zhou W, Wang Y, Sun Z, Wu X, Chen S, Ni M, Hu Y. Mobile plasmid mediated transition from colistin-sensitive to resistant phenotype in Klebsiella pneumoniae. Front Microbiol. 2021;12:619369.

32. Jayol A, Nordmann P, Brink T, Villegas M-V, Dubois V, Poirel L. High-level resistance to colistin mediated by various mutations in the mgrB gene among carbapenemase-producing Klebsiella pneumoniae. Antimicrob Agents Chemother. 2017;61(11):e01423-e01417.

33. McConville TH, Annavajhala MK, Giddins MJ, Macieszczak N, Herrera CM, Rozenberg FD, Bhusan GL, Ahn D, Manica F, Trent MS. CfrB positively regulates high-level polymyxin resistance and virulence in Klebsiella pneumoniae. Cell reg. 2020;33(4):108313.

34. Aires CAM, Pereira PS, Avesani MD, Carvalho-Assaf APDA. mgrB mutations mediating polymyxin B resistance in Klebsiella pneumoniae isolates from rectal surveillance swabs in Brazil. Antimicrob Agents Chemother. 2016;60(11):6969–72.

35. Choi M, Ko KS. Identification of genetic alterations associated with acquired colistin resistance in Klebsiella pneumoniae isogenic strains by whole-genome sequencing. Antimicrob Agents Chemother. 2020;64(7):e00981-00919.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.