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Genome Note

Genome characterization of a *Klebsiella pneumoniae* co-producing OXA-181 and KPC-121 resistant to ceftazidime/avibactam, meropenem/vaborbactam, imipenem/relebactam and cefiderocol isolated from a critically ill patient

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ABSTRACT

Objectives: : Carbapenemase-producing Enterobacterales (CPE) represent a public health concern. The limited antimicrobial options against CPE have led to the development of novel antimicrobial molecules. In the present study, we characterized the genetic determinants associated with the resistance to ceftazidime/avibactam (CAZ-AVI), meropenem/vaborbactam (MER-VAB), imipenem/relebactam (IMI-REL) and cefiderocol (CFD) in a carbapenemase-producing *Klebsiella pneumoniae* strain isolated from a critically ill patient.

Methods: : Genomic DNA was sequenced using Illumina iSeq 100 and Minion Oxford Nanopore platforms. Assemblies were performed with a de novo approach using short-read, hybrid and long-lead assembly approaches. Final assembly was manually curated and carefully verified. Circular elements were screened for antimicrobial-resistance genes, porins, virulence factors and prophage regions.

Results: : KPC-Kp (KPC-producing *Klebsiella pneumoniae*) BO743 was resistant to all novel β-lactams including CAZ-AVI, MER-VAB, IMI-REL and CFD. The genome of strain BO743 is composed of a single chromosome of 5 347 606 bp and three circular plasmids of 363 634 bp (pBO743-363Kb), 120 290 bp (pBO743-120Kb) and 54 339 bp (pBO743-54Kb). Sequence analysis demonstrated that KPC-Kp BO743 co-harboured $bla_{OXA-181}$ and novel $bla_{KPC-121}$ located, respectively, on the pBO743-54Kb and pBO743-120Kb plasmids. KPC-121 differed by a serine insertion at position 181 than KPC-3.

Conclusion: : The description of the genome of KPC-Kp cross-resistant to novel βL-βLICs and cefiderocol reveals the presence of numerous antimicrobial resistance genes including $bla_{OXA-181}$ and novel variant $bla_{KPC-121}$. The characterization of this multidrug-resistant phenotype provides evidence that needs further attention and monitoring of such MDR clinical isolates.

1. Introduction

In the last years, the prevalence of carbapenemase-producing Enterobacterales (CPE) has been increasingly reported worldwide, thus resulting in a serious threat for public health [1]. Novel β-lactam/β-lactamase inhibitor combinations (βL-βLICs) and siderophore cephalosporin, cefiderocol (CFD), were recently developed to overcome the lack of effective antimicrobial molecules for treatment of infections due to CPE [2]. Although novel βL-βLICs and CFD partially solved the paucity of antimicrobial treatments against KPC-Kp, the recent emergence of clinical strains resistant to CFD, ceftazidime/avibactam (CAZ-AVI) and/or meropenem/vaborbactam (MER-VAB) and/or imipenem/relebactam (IMI-REL) mitigated their potential clinical impact [3,4].

Here, we describe the genome of a *K. pneumoniae* co-producing KPC-121 and OXA-181 carbapenemase cross-resistant to CAZ-AVI, MER-VAB, IMI-REL and CFD.

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Table 1
Antimicrobial susceptibility of the KPC-producing *K. pneumoniae* strain BO743

Antimicrobials	MIC (mg/L)
Amoxycillin/clavulanate	≥32
Ceftazidime	≥256
Ceftriaxone	≥256
Ertapenem	≥256
Meropenem	16
Imipenem	8
Ceftazidime/Avibactam	≥256
Meropenem/Vaborbactam	32
Imipenem/Relebactam	8
Cefiderocol[a]	8
Colistin[b]	0.125
Gentamicin	8
Amikacin	16

[a] MIC determined using iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB).
[b] MIC confirmed by broth microdilution (BMD) assay.

2. Material and methods

The *K. pneumoniae* BO743 was isolated from a central-venous catheter (CVC) of a critically ill patient hospitalized at St. Orsola-Malpighi University-Hospital in Bologna, Italy on 16 April 2021. Antimicrobial susceptibility testing was performed using a EUMDROXF® plate (Thermo Fisher Scientific, USA), and MICs for novel βL-βLICs were confirmed using MIC test strips (Liofilchem, Italy), while MIC for CFD was performed as previously described [5]. MIC results were interpreted following EUCAST clinical breakpoints v.11.0 (available at: https://www.eucast.org/clinical_breakpoints/).

Genomic DNA was sequenced using both Illumina iSeq 100 (Illumina, San Diego, CA, USA) and Oxford Nanopore MinION (Oxford Nanopore Technologies, UK) platforms. Short-reads de novo assembly was performed using Spades v.3.15.4, and a hybrid approach was performed using Unicycler v.0.5.0, while long-reads assemblies were performed using Flye v.2.3.1 and Canu v.1.9. Long-reads assembled contigs were polished with Illumina reads using Polypolish v.0.5.0. Final assembly was obtained by comparing different approaches, thus confirming circularity of sequence contigs and uniform coverage of Illumina and Nanopore mapped reads. MLST and resistome analysis were performed using CGE server (https://www.genomicepidemiology.org), and ß-lactamase content was confirmed by using BLAST analysis against CARDB and Beta-Lactamase Database (http://bldb.eu). Porin genes were manually investigated by BLAST analysis against reference proteins (OmpK35 [O87753], OmpK36 [D6QLX8], OmpK37 [S5UDN6]), and prophage regions within the KPC-Kp genome were assessed using the PHASTER web tool (https://phaster.ca). The circular representation of plasmids was *generated* using *BRIG v.0.95.*

3. Results

The antimicrobial susceptibility profile of Kp BO743 is shown in Table 1. Kp BO743 exhibited a high level of resistance to all β-lactams including novel βL-βLICs (CAZ-AVI, MER-VAB, IMI-REL), CFD and aminoglycosides, while it was susceptible only to colistin (Table 1).

A total of 1 453 875 Illumina high-quality reads and 260 101 Nanopore long-reads with an average length of 5542 bp (ranging from 115 to 76 836 bp) were generated. The final assembly generated a singular chromosome of 5 347 606 bp and three circular plasmids named pBO743-363Kb (363 634 bp), pBO743-120Kb (120 290 bp) and pBO743-54Kb (54 339 bp) (Fig. 1). Annotation gene prediction identified a total of 6339 genes including 5916 protein-coding. Genetic analysis demonstrated that KPC-Kp strain BO743 belonged to ST512 and harboured the CPS cluster genes *wzi-154* and *wzc-916*. Resistome analysis demonstrated that strain BO743 harboured a total of six genes conferring resistance to β-lactams including $bla_{\text{OXA-181}}$ and $bla_{\text{KPC-121}}$ carbapenemase, five for aminoglycosides, three for fluoroquinolones, two sulphonamides and two for trimethoprim, most of them located on different plasmids (Supplementary Table S1). Amino acid sequence showed that KPC-121 exhibited a duplication of a serine at position 181 (duplication of TCA) in comparison to KPC-3 (Supplementary Fig. S1). This insertion occurred close to the Ω-loop of KPC enzyme, a region that has been demonstrated to be an important active site of β-lactamase and frequently associated with mutations related to CAZ-AVI-resistance [3].

Genomic analysis revealed that the bla_{KPC} and $bla_{\text{OXA-181}}$ genes were located, respectively, on pBO743-120Kbp and pBO743-54Kb plasmids. Blast analysis showed that pBO743-120Kbp exhibited high homology to pIT-1825-FIIK1, a plasmid carrying KPC-53-variant associated to CAZ-AVI resistance reported in Italy (acc. no. CP058327) [6], while BO743-54Kb showed high nucleotide identity to plasmids carrying $bla_{\text{OXA-181}}$ gene isolated in several countries (e.g., pLB_OXA-181_PT109 [Acc-no. NZ_CP041033] isolated in Portugal and pC872_2[NZ_CP067461] isolated in the United States).

Analysis of the prophage regions revealed that BO743 strain carried a total of 15 prophage sequences including five intact, six incomplete and four questionable regions mostly distributed on chromosome and plasmid pBO743-363Kb. Deep genome analysis demonstrated that different virulence factors including iron acquisition systems (*iutA)* and type 3 fimbries (*mrk* operon) were located on chromosome outside prophage regions (Supplementary Table S2).

4. Conclusion

Here we describe the genome of a *K. pneumoniae* strain BO743 resistant to CFD, CAZ-AVI, MER-VAB and IMI-REL. The KPC-kp strainBO743 harboured several antimicrobial resistance genes, including $bla_{\text{OXA-181}}$ and novel $bla_{\text{KPC-121}}$, which is associated to the high level of resistance to CAZ-AVI. Virulome analysis demonstrated that the different virulence factors were carried on the chromosome of KPC-Kp strain BO743 outside the prophage regions, thus confirming a stable diffusion of virulent CC258 KPC-Kp clone in our region. These findings are in accordance with a previous study showing that successful clonal lineage (ST258/512) co-evolved stably pKpQIL-like and IncX3 plasmids carrying bla_{KPC} gene [7]. At the same time, we demonstrated that KPC-Kp strain BO743 acquired an epidemic pOXA-48–like plasmid, thus conferring cross-resistance to novel molecules βL-βLICs and CFD due to the co-production of OXA-181 and KPC-121 carbapenemase. Based on these data, further attention must be paid to monitor and reduce the transmission and possible diffusion of such a MDR clone in clinical settings.

GenBank accession numbers

The whole complete genome sequences of Klebsiella pneumoniae strain BO743 was deposited in GenBank (Bioproject no. SAMN27596901) under accession no. CP095780, CP095781, CP095782, CP095783.

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Ethical approval

Not required.

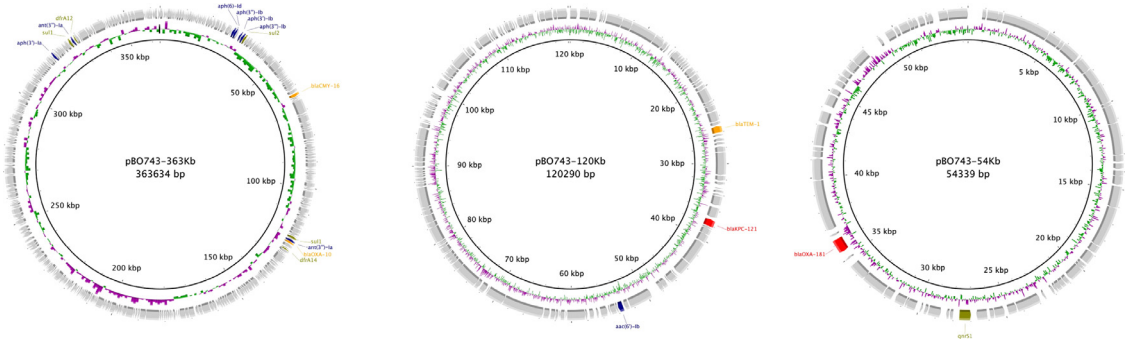

Fig. 1. Circular representation of circular plasmids pBO743-363Kb, pBO743-120Kb and pBO743-54Kb in *Klebsiella pneumoniae* strain BO743. The CDS are indicated by arrowheads, and coding sequences conferring antimicrobial resistances are colour-coded (CDS conferring resistance to β-lactams are displayed in orange, carbapenem in red, aminoglycosides in blue and others in green). The innermost circle represents the GC skew (purple/green).

Competing interests

None declared.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.06.021.

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