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Original Article

Genomic and prophages analysis of a ST16 *Klebsiella pneumoniae* carrying *bla*$_{OXA-181}$ from Botswana, 2023

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Abstract

Introduction: Carbapenemase-producing Enterobacterales (CPE) pose a serious threat to public health worldwide and are a challenge for clinicians due to the limited antimicrobial options available. Here, we characterize the genome of a *Klebsiella pneumoniae* (named Kp51) producing OXA-181 carbapenemase isolated from a rectal swab of a hospitalized patient in Botswana in 2023. We also investigate the genomic epidemiology of carbapenemase gene and prophage regions among *bla*$_{OXA-181}$-harboring *K. pneumoniae* strains collected worldwide.

Methodology: Whole-genome sequencing was performed on the Illumina MiSeq system, and assembly was executed with SPAdes. Phylogenetic analysis was performed on core genome SNPs among OXA-181-producing *K. pneumoniae* genomes. Analysis of prophage regions was performed among closely related genomes by pairwise comparison.

Results: Resistome analysis results showed that Kp51 harbored qnrS1, *bla*$_{TEM-1}$, and *bla*$_{OXA-181}$ carbapenemase gene. Also, Kp51 strain belonged to ST16 and carried an IncX3 plasmid harboring the *bla*$_{OXA-181}$ carbapenemase gene. Phylogenetic analysis showed that the Kp51 genome was closely related to an OXA-181-producing strain isolated from a dog in China, and the IncX3 plasmid exhibited high sequence homology with *bla*$_{OXA-181}$-carrying plasmids collected worldwide. Prophage analysis revealed that high variability was observed in ST16 *K. pneumoniae* strains harboring *bla*$_{OXA-181}$.

Conclusions: Here we demonstrate the genomic spread of OXA-181-producing ST16 *K. pneumoniae* worldwide and that the major source of variability is located within prophage regions. Our work emphasizes the need for constant monitoring of the OXA-181 carbapenemase producers diffusion with special emphasis on low and middle-income countries in order to trace the spread of CPE pathogens globally.

Key words: *bla*$_{OXA-181}$; Botswana; prophages; epidemiology.

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Introduction

Resistance to carbapenems has rapidly risen in the past two decades, prompting the World Health Organization to recognize Carbapenemase-Producing Enterobacterales (CPE) as a critical healthcare concern for which new antibiotics are urgently needed [1]. Indeed, infections caused by CPE have been associated with increased mortality, longer hospital stays, and higher hospital costs [2-3].

The global spread of CPE is due to multifactorial reasons, including inappropriate antibiotic use, poor/ineffective infection control, as well as the interconnectedness of healthcare systems worldwide. Epidemiology of carbapenemase genetic determinants exhibits high variability of different carbapenemase genes by showing different endemic circulation among different countries [4]. Despite the paucity of data, several carbapenemase-encoding genes have been characterized from samples identified in Africa. In particular, *bla*$_{NDM}$ and *bla*$_{OXA-48}$ were the most prevalent carbapenemase-encoding genes in Southern Africa, although the burden and characterization of CPE are still lacking in several African countries [5-7]. This epidemiology is consistent across different studies from South Africa (i.e., *bla*$_{NDM}$ and *bla*$_{OXA-48}$-like) and Angola (i.e. *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$; *bla*$_{OXA-181}$), where there are at least two studies reporting the genomic characterization of CPE [8-9].

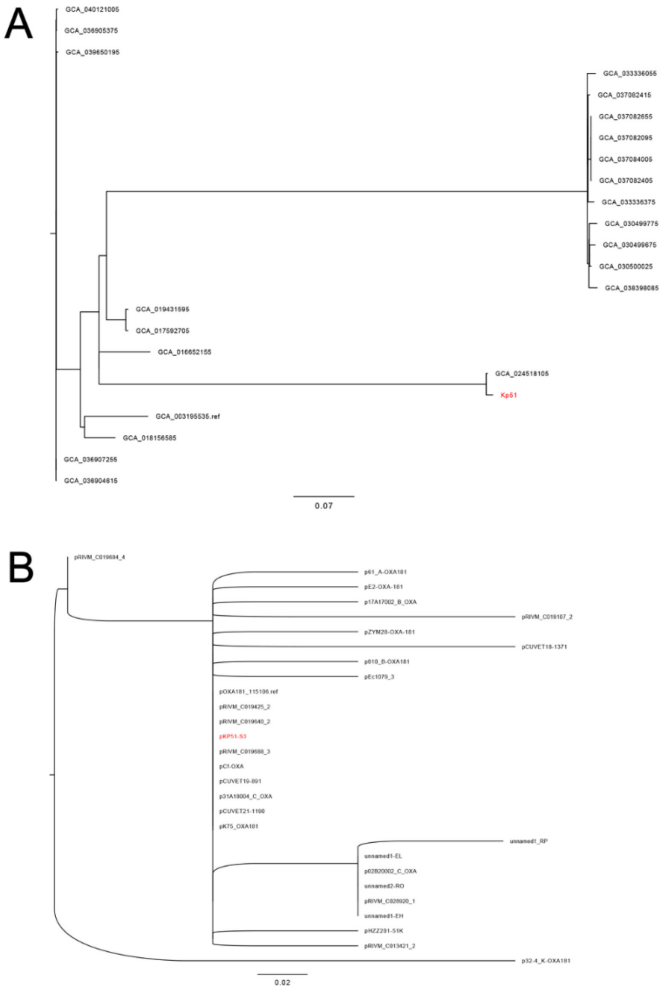
Based on these considerations, we characterized the genetic architecture of a *Klebsiella pneumoniae*

carrying *bla*$_{OXA-181}$ collected from a patient in Botswana, and contextualized its genome in comparison to available closer genomes collected worldwide. In order to define the worldwide spreading of plasmids harboring *bla*$_{OXA-181}$ and to define the role of prophages in genomic variability among *K. pneumoniae,* we identified and compared the prophage regions within ST16 *K. pneumoniae* genomes harboring *bla*$_{OXA-181}$ and defined their genomic context.

Methodology

The Kp51 strain was isolated from a rectal swab collected from a patient hospitalized in Botswana in 2023. Species identification was performed using MALDI-TOF Biotyper (Bruker, Massachusetts, US) system. Antimicrobial susceptibility testing was performed using both Vitek2 and Sensititre Gram-negative MDRGN1F panel (Thermofisher, MS, USA). DNA extraction and library preparation were performed as previously described [10]. Briefly, DNA Sequencing was conducted on the Illumina MiSeq instrument (Illumina, California, USA) system and the genome was assembled with SPAdes v3.15.2 MLST and resistome analysis was investigated using AMRFinderPlus v3.11.14 (https://github.com/ncbi/amr). Genomic relationships were performed based on core genome SNPs analysis and the list of genomes used for phylogenetic analysis is shown in Supplementary Table 1.

Figure 1 A. Phylogenetic analysis based on the core genome SNPs of the ST16 *Klebsiella pneumoniae* genomes carrying blaOXA-181. The K51 strain is highlighted in red. **B.** Phylogenetic tree of IncX plasmids carrying *bla*$_{OXA-181}$ carbapenemase collected worldwide. The scale bar represents substitutions per site.



Analysis of the prophage regions was performed as previously described [11]. Briefly, prophages were identified by comparing the genome of the Kp51 strain to prophages present in the software database and assigning a score based on the coverage of phage organisms (http://phastest.ca). The prophage regions for each strain were extracted, and pairwise prophage comparison was performed with ANI analysis.

Plasmid replicon types were determined with PlasmidFinder v2.1.6 (https://cge.food.dtu.dk/services/PlasmidFinder/). Phylogenetic correlation with plasmid sequences available in GenBank was performed using the Harvest suite (https://harvest.readthedocs.io/en/latest/). The list of plasmids used for sequence comparison is shown in Supplementary Table 2.

Results

Antimicrobial susceptibility testing revealed that the Kp51 strain was susceptible to ceftazidime/avibactam, imipenem/relebactam, meropenem/vaborbactam, aztreonam, meropenem, cefepime and ceftolozane/tazobactam, while was resistant to eravacycline and amikacin. Antimicrobial susceptibility pattern of Kp51 clinical isolate was shown in Supplementary Table 3.

Genome-based typing revealed that Kp51 belonged to the ST16 and harbored the KL51 (K-locus) and the wzi50 (capsule repeat unit polymerase) alleles. Analysis of the antimicrobial resistance determinants showed that the Kp51 strain carried different determinants, including resistance to β-lactams [*bla*SHV-1, *bla*$_{OXA-181}$] and quinolones [qnrS1]. At the same time, genetic determinants related to resistance to other classes of antimicrobials have not been found within the genome of the Kp51 strain. Genomic statistics of the Kp51 strain are shown in Table 1.

The genomic relatedness of the Kp51 strain with other ST16 *K. pneumoniae* strains collected worldwide was contextualized through a phylogenetic

reconstruction. The antimicrobial genetic determinants, serotype, data, and country of collection for each strain used for genome comparison are shown in Supplementary Table 1. The phylogenetic tree revealed that Kp51 clustered closely to the genome of a ST16 *K. pneumoniae* strain harboring $bla_{OXA-181}$ carbapenemase isolated from a urine specimen collected from a dog in China in 2018 (Figure 1 A). In detail, genome comparison with the D15P156 strain showed that Kp51 exhibited an OrthoANIu value of 99.98% with an average aligned length (bp) of 4.263.195 bp (total genome length of Kp51 was 5.371.320 bp).

Plasmid content analysis demonstrated that the Kp51 strain harbored the ColKP3, IncFIB(K), IncX3 plasmid Inc types. Genomic analysis revealed that the QnrS1 and $bla_{OXA-181}$ resistance genes were located within a plasmid of 50.165 bp belonging to the IncX3 type. *Bla*st analysis demonstrated that the IncX3 plasmid carrying $bla_{OXA-181}$ of the Kp51 strain exhibited a common genetic backbone [100% coverage and 99.99% identity] to several $bla_{OXA-181}$ carrying plasmids isolated from different bacterial spp. in several countries [i.e., UK, Netherlands, China, etc.]. Phylogenetic analysis performed on the plasmids carrying a similar genetic backbone demonstrated that the plasmid carrying $bla_{OXA-181}$ carbapenemase of Kp51 strain, named K51-S3, was closely related to plasmids isolated in China, the Netherlands, Italy, Canada, and Thailand (Figure 1 B) from different bacterial spp.

To evaluate the role of the prophages in the *K. pneumoniae* population and define their genomic distribution, we performed sequence analysis in the genomes of the Kp51 clinical isolate and contextualized these regions in comparison to the ST16 *K. pneumoniae* strains carrying $bla_{OXA-181}$ collected worldwide. Our analysis demonstrated that Kp51 strain harbored 4 intact prophage regions within the chromosome with a sequence length of 64.3, 45.8, 38.3, and 36.5 Kb (Figure 2 A).

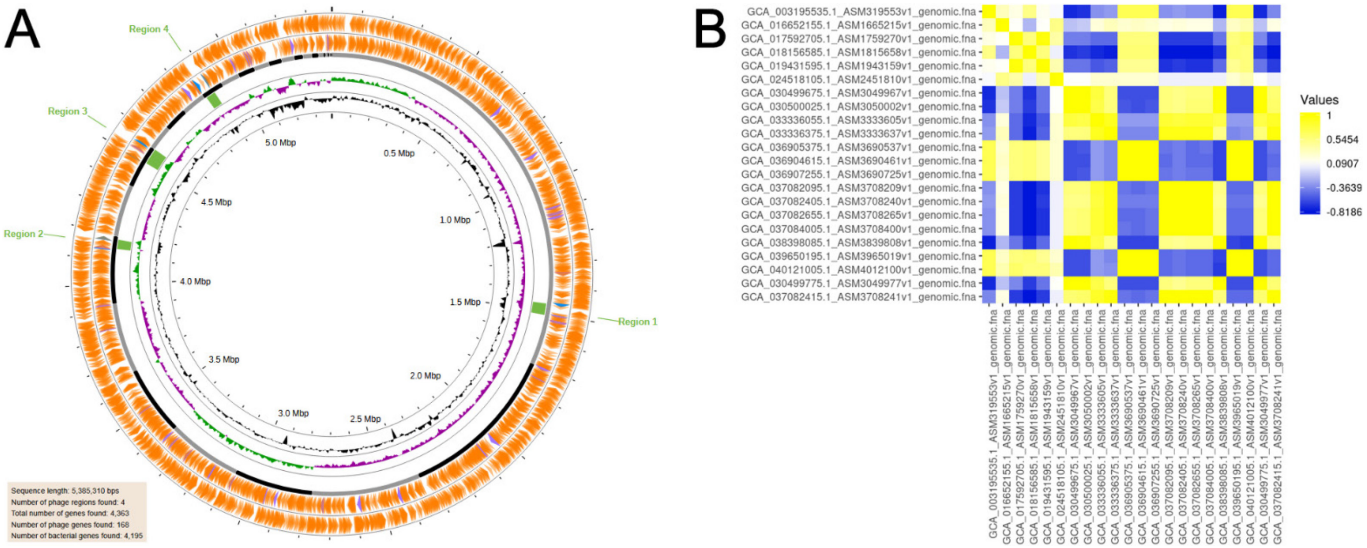
Table 1. Genomic characteristics of the blaOXA-181-carrying ST16 K. pneumoniae strain Kp51 included in this study.

Genome statistics	Kp51
Total Length (bp)	5.385.310
No. of Contigs	32
N50 (bp)	426.292
N90 (bp)	93.192
L50	3
L90	13
G + C (%)	57.29
No. of predicted genes	5.410

Genome analysis of the ST16 *K. pneumoniae* strains carrying $bla_{OXA-181}$ showed that intact prophage regions ranged from 15 Kb to 110 Kb (median 38 Kb, IQR 29-47 Kb), while the number of these regions ranged from 4 Kb to 8 Kb (median 5 Kb, IQR 4-7 Kb), (Supplementary Figure 1).

To investigate the evolutionary relationship among prophage regions within ST16 *K. pneumoniae* strains carrying $bla_{OXA-181}$, pairwise homology comparison was performed among prophage sequences. Our results demonstrated that 16% (74 out of 462) showed a sequence homology higher than 80% among prophages, thus demonstrating a high variability among prophage regions in ST16 *K. pneumoniae* strains harboring $bla_{OXA-181}$ carbapenemase (Figure 2 panel B). Sequence

Figure 2 A. Graphical representation of the chromosome of the ST16 *Klebsiella pneumoniae* strain Kp51. The coding sequences (CDS) and pro-phage regions are colored in orange and green respectively. **B.** Heatmap of pairwise ANI values in the prophages regions among ST16 *Klebsiella pneumoniae* strains carrying $bla_{OXA-181}$ carbapenemase



analysis showed that no antimicrobial resistance genes were located within the prophage regions of the ST16 *K. pneumoniae* genomes harboring *bla*$_{OXA-181}$.

Conclusions

In conclusion, here we characterized the genome of a *bla*$_{OXA-181}$ -carrying *K. pneumoniae* Kp51 strain in Botswana in 2023. Our results demonstrated that the resistome of Kp51 was composed by a few antimicrobial resistance genes, including determinants related to the resistance to fluoroquinolones and B-lactams, while no genes related to other classes of antimicrobials (i.e., aminoglycosides, colistin, Fosfomycin, sulfonamides, and tetracycline) were observed. Also, we observed that the Kp51 strain was highly susceptible to novel β-lactam/β-lactamase inhibitor combinations [BL-BLICs] (i.e., ceftazidime/avibactam, meropenem/vaborbactam etc.), thus suggesting their potential use in the treatment of infections due to OXA-181 producers.

Genomic analysis showed that the Kp51 strain genome was closely related to different ST16 *K. pneumoniae* genomes harboring *bla*$_{OXA-181}$ collected worldwide. At the same time, the high nucleotide homology of *bla*$_{OXA-181}$ plasmids collected from different countries suggested a broad circulation of clonally related strains globally. Also, we demonstrated that most of the genetic differences among ST16 *K. pneumoniae* genomes harboring *bla*$_{OXA-181}$ carbapenemase were located within prophage regions. In particular, we observed a marked variability of the prophage numbers and a low degree of conservation among different ST16 *K. pneumoniae* strains, thus suggesting that the integration of prophages within CPE genomes could play a key role in the differences of clonally related strains. At the same time, we observed that no antimicrobial resistance determinants were found within prophages, thus suggesting no effective role of these regions in antimicrobial gene acquisition on the ST16 *K. pneumoniae.*

In conclusion, our study aimed to characterize the OXA-181-producing ST16 *K. pneumoniae* in Botswana by providing a genomic analysis of prophages circulating in this genotype. In this context, the increasing CPE circulation in low- and middle-income countries reinforces the necessity of an active CPE surveillance, especially in these regions and suggests the application of novel sequencing technologies for genomic investigations. Our study highlights the importance of characterizing the genomes of CPE in low and middle-income countries to monitor the spread of MDR pathogens and resistance determinants by rapidly identifying them and tracing possible emerging resistance genotypes.

Acknowledgements

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Data availability

The genomic sequence of Kp51 strain was deposited in the NCBI database under the Bioproject PRJNA1236353, Biosample SAMN47385852, and Assembly Acc.no JBMDMF000000000.

Authors contribution

PG, GMP, Conceptualization, Study design, Data collection, Writing - Original Draft, Writing - Review & Editing, and Supervision; PN Data collection, Data analysis, Experiment, Writing - Original Draft; JPS and OM Review & Editing and Supervision.

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Conflict of interest

No conflict of interest is declared.

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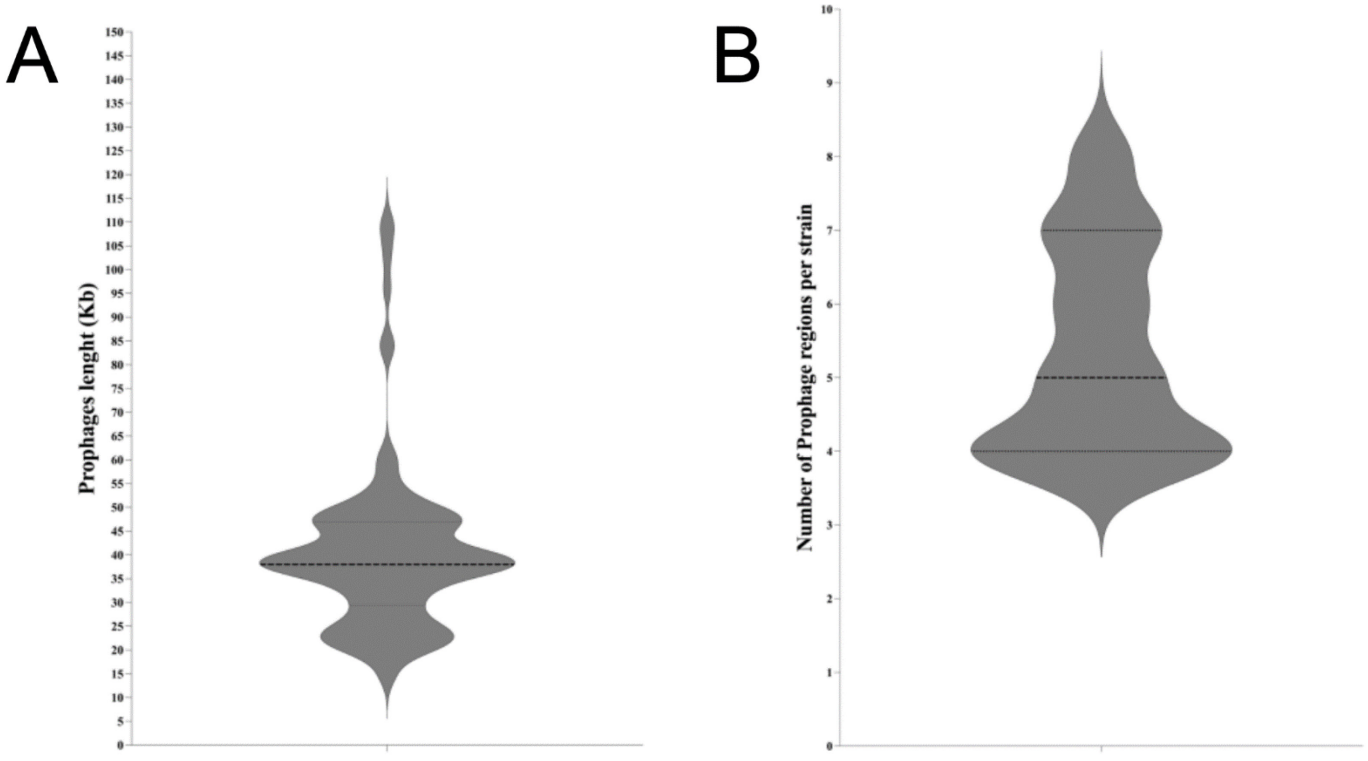
Annex – Supplementary Items

Supplementary Table 1. Genomic characteristics of *bla*$_{OXA-181}$-carrying ST16 *Klebsiella pneumoniae* strains collected worldwide.

Accession number (Strain)	ST	K-locus	Collection date	Country	Host	*wzi*	Antimicrobial Resistance Determinants									Porins
							Aminoglycosides	Colistin	Fosfomycin	Fluoroquinolone	Sulfonamides	Tetracycline	Beta-Lactamases (bla)	Extended Spectrum Beta-Lactamases (bla)	Carbapenemase	
JBMDMF000000000/Kp51	16	KL51	2023	Botswana	*Homo sapiens*	*wzi50*	-	-	-	qnrS1	-	-	-	-	SHV-1	OXA-181	-
GCA_003195535.1	16	KL51	2017	USA	*Homo sapiens*	*wzi50*	aac(6')-Ib; aadA5; rmtF	-	-	qnrS1	sul1	-	-	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_016652155.1	16	KL51	NI	Egypt	*Homo sapiens*	*wzi50*	aac(6')-Ib-cr; strA; strB	-	-	qnrS1	sul2	-	OXA-1	CTX-M-14; CTX-M-15	SHV-1	OXA-181	-
GCA_017592705.1	16	KL51	NI	NI	NI	*wzi50*	rmtB	-	-	qnrS1	-	-	TEM-1D	-	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_018156585.1	16	KL51	NI	NI	NI	*wzi50*	aac(6')-Ib; aadA; rmtF	-	-	-	sul1	-	OXA-9; TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_019431595.1	16	KL51	2019	China	*Homo sapiens*	*wzi50*	rmtB	-	-	qnrS1	-	-	TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_024518105.1	16	KL51	2018	China	Dog	*wzi50*	-	-	-	qnrS1	-	-	-	-	SHV-1	OXA-181	-
GCA_030499675.1	16	KL81	2021	India	*Homo sapiens*	*wzi230*	aac(6')-Ib-cr; strA; strB	-	-	qnrS1	sul2	-	OXA-1; TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated
GCA_030499775.1	16	KL81	2021	India	*Homo sapiens*	*wzi230*	aac(3)-IIa; aac(6')-Ib-cr; strA; strB	-	-	qnrS1	sul2	-	OXA-1	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_030500025.1	16	KL81	2021	India	*Homo sapiens*	*wzi230*	aac(6')-Ib-cr; strA; strB	-	-	-	sul2	-	OXA-1	-	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_033336055.1	16	KL81	2022	Uganda	*Homo sapiens*	*wzi230*	rmtB	-	-	qnrS1	-	-	TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_033336375.1	16	KL81	2021	Uganda	*Homo sapiens*	*wzi230*	rmtB	-	-	qnrS1	-	-	TEM-1D	-	SHV-1	OXA-181	OmpK35_truncated
GCA_036904615.1	16	KL51	2023	USA	*Homo sapiens*	*wzi50*	aac(6')-Ib-cr; aadA16; rmtB	-	-	qnrB6; qnrS1	sul1	tetA	-	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_036905375.1	16	KL51	2023	USA	*Homo sapiens*	*wzi50*	aac(6')-Ib-cr; aadA16; rmtB	-	-	qnrB6; qnrS1	sul1	tetA	-	CTX-M-15	SHV-1	OXA-181	OmpK35_truncated; OmpK36_truncated
GCA_036907255.1	16	KL51	2023	USA	*Homo sapiens*	*wzi50*	aac(6')-Ib-cr; aadA16; rmtB	-	-	qnrB6; qnrS1	sul1	tetA	TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35-8%; OmpK36TD
GCA_037082095.1	16	KL81	2020	India	*Homo sapiens*	*wzi230*	aac(3)-IId; aadA; rmtB	-	-	qnrS1	sul1	-	-	CTX-M-15	-	OXA-181	OmpK35-6%; OmpK36TD
GCA_037082405.1	16	KL81	2021	India	*Homo sapiens*	*wzi230*	aac(3)-IId; aadA; rmtB	-	-	qnrS1	sul1	-	TEM-1D	CTX-M-15	-	OXA-181	OmpK35-6%; OmpK36TD
GCA_037082415.1	16	KL81	2020	India	*Homo sapiens*	*wzi230*	aac(3)-IId; aadA; rmtB	-	-	qnrS1	-	-	TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35-6%; OmpK36TD
GCA_037082655.1	16	KL81	2023	India	*Homo sapiens*	*wzi230*	aac(3)-IId; aadA; rmtB	-	-	qnrS1	sul1	-	TEM-1D	CTX-M-15	-	OXA-181	OmpK35-6%; OmpK36TD
GCA_037084005.1	16	KL81	2021	India	*Homo sapiens*	*wzi230*	aac(3)-IId; aadA; rmtB	-	-	qnrS1	sul1	-	-	CTX-M-15	-	OXA-181	OmpK35-6%; OmpK36TD
GCA_038398085.1	16	KL81	2022	USA	*Homo sapiens*	*wzi230*	aac(3)-IIa; aac(6')-Ib-cr; aadA; aph(3')-Ia; strA; strB	-	-	-	sul2; sul3	-	OXA-1; TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35-6%; OmpK36TD
GCA_039650195.1	16	KL51	2024	USA	*Homo sapiens*	*wzi50*	rmtB	-	-	qnrS1	-	-	-	CTX-M-15	SHV-1	OXA-181	OmpK35-8%; OmpK36TD
GCA_040121005.1	16	KL51	2024	USA	*Homo sapiens*	*wzi50*	aac(6')-Ib-cr; aadA16; rmtB	-	-	qnrB6; qnrS1	sul1	tetA	TEM-1D	CTX-M-15	SHV-1	OXA-181	OmpK35-8%; OmpK36TD

Supplementary Figure 1. Prophages distribution among *bla*$_{OXA-181}$-carrying ST16 *K. pneumoniae* genomes. **A.** length of prophage regions; **B.** number of prophage regions per strain.



Supplementary Table 2. List of *bla*$_{OXA-181}$-carrying plasmids collected from GenBank.

Plasmid	Acc.no.	Organism	Host	Country	Date	ST	Sample
KP75_L14_02.23_ST20_OXA181	CP141549.1	*Klebsiella pneumoniae*	Human	UK	2023	2096	Blood
pRIVM_C019640_2	CP068881.1	*Escherichia coli*	Human	Netherlands	2019	-	-
pRIVM_C019425_2	CP068910.1	*Escherichia coli*	Human	Netherlands	2019		
p010_B-OXA181	CP048332.1	*Escherichia coli*	environment	Switzerland			
pRIVM_C019684_4	CP068959.1	*Klebsiella pneumoniae*	Human	Netherlands	2019		
pEc1079_3	CP081309.1	*Escherichia coli*	Human	Ghana	2015		Urine
p17A17002_B_OXA	PP320286.1	*Escherichia coli*	Human	Canada	2017		rectal swab
pE2-OXA-181	CP048918.1	*Escherichia coli*	Human	Egypt	2015		ascitic fluid
p61_A-OXA181	CP048327.1	*Escherichia coli*	environment	Switzerland	2019		
pRIVM_C013421_2	CP068328.1	*Escherichia coli*	Human	Netherlands	2019		
unnamed2-RO	CP166146.1	*Raoultella ornithinolytica*	Human	USA	2022		Other
pCf-OXA	CP110780.1	*Citrobacter freundii*	Human	Italy	2020		Rectal swab
pRIVM_C028920_1	CP068938.1	*Escherichia coli*	Human	Netherlands	2019		
pHZZ201-51K	CP168040.1	*Escherichia coli*	pig	China	2023		
pKP444-1	CP166765.1	*Escherichia coli*	Human	Taiwan	2019		
pRIVM_C019107_2	CP068919.1	*Klebsiella pneumoniae*	Human	Netherlands	2019		
pRIVM_C019688_3	CP068958.1	*Klebsiella pneumoniae*	Human	Netherlands	2019		
pCUVET19-891.3	CP114988.1	*Enterobacter hormaechei*	Human	Thailand	2019		Urine
pOXA181_115106	CP043335.1	*Escherichia coli*	Human	China	2017		
p32-4_K-OXA181	CP048321.1	*Escherichia coli*	environment	Switzerland	2019		
p02B20002_C_OXA	PP320251.1	*Raoultella ornithinolytica*	Human	Canada	2020		urine
pCUVET21-1190.1	CP114995.1	*Enterobacter hormaechei*	Human	Thailand	2021		abdominal fluid
unnamed1-RP	CP174429.1	*Raoultella planticola*	Human	USA	2024		urine
p21C20004_A_OXA	PP320289.1	*Citrobacter werkmanii*	Human	Canada	20'20		urine
unnamed1-EL	CP131963.1	*Enterobacter ludwigii*	Human	USA	2023		urine
pCUVET18-1371.4	CP115013.1	*Serratia nevei*	Dog	Thailand	2018		wound
pNUITM-VK5_mdr	LC633285.1	*Klebsiella aerogenes.1*	-	Viet Nam	2021		
p31A18004_C_OXA	PP320301.1	*Klebsiella oxytoca*	Human	Canada	2018		rectal swab
pZYM28-OXA-181	MW080368.1	*Morganella morganii*	Human	China	2018		shunt fluid
unnamed1-EH	CP104690.2	*Enterobacter hormaechei*	Human	USA	2020		urine

Supplementary Table 3. MIC values for all compounds tested on strain *bla*$_{OXA-181}$-carrying ST16 *K. pneumoniae* strain Kp51, and respective interpretation according to the latest EUCAST guidelines (v15.0) (https://www.eucast.org/clinical breakpoints).

Antibiotic	MIC (µg/ml)	MIC Interpretation
amikacin	16	resistant
aztreonam	< 1	susceptible
cefepime	< 1	susceptible
ceftazidime/avibactam	< 0.25	susceptible
ceftolozane/tazobactam	0.5	susceptible
eravacycline	0.25	resistant
imipenem	< 1	susceptible
imipenem/relebactam	0.25	susceptible
meropenem	1	susceptible
meropenem/vaborbactam	0.5	susceptible
piperacillin/tazobactam	> 32	resistant
tigecyclin	< 0.5	NA
colistin	< 0.5	susceptible
tobramycin	< 0.5	susceptible

MIC results were interpreted following EUCAST guidelines (v15.0). NA: Not Applicable.