Genomic Characterization of a Multidrug-Resistant and Hypermucoviscous/Hypervirulent *Klebsiella quasipneumoniae* subsp. *similipneumoniae* ST4417 Isolated from a Sewage Treatment Plant

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Aim: Clinical strains of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* have been reported worldwide. Multidrug-resistant (MDR) hypermucoviscous (hm)/hypervirulent (hv) lineages have become a global problem for public health worldwide. Therefore, this study aimed to characterize by whole-genome sequencing a MDR-hm/hv *K. quasipneumoniae* subsp. *similipneumoniae* SWT10 strain belonging to the new sequence type ST4417 isolated from a sewage treatment plant.

Results: The SWT10 strain was recovered from a sewage treatment plant in Brazil and presented the hm and MDR phenotypes. Resistome analysis showed antimicrobial resistance genes associated with resistance to fluoroquinolones, β -lactams, tetracyclines, trimethoprim, aminoglycosides, sulfonamides, macrolides, and fosfomycin as well as several heavy metal resistance genes. Virulome analysis showed virulence factors related to hv lineages. Multilocus sequence typing analysis revealed the new ST4417, which was grouped in CC1584 by the minimum-spanning tree. Besides, five plasmid incompatibility groups, two prophage-related sequences, and 66 genomic islands were detected.

Conclusion: This study reports for the first time the genome sequence of a MDR-hm/hv *K. quasipneumoniae* subsp. *similipneumoniae* recovered from the environment, which contributes to a better understanding about these lineages as well as for surveillance studies worldwide.

Keywords: *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, multidrug-resistant, hypermucoviscous, hypervirulent, water

Introduction

KLEBSIELLA QUASIPNEUMONIAE SUBSP. *similipneumoniae*, also termed as belonging to the phylogenetic group KpIIb of *Klebsiella pneumoniae*, has been reported worldwide, mainly in clinical sources. Hypermucoviscous (hm)/hypervirulent (hv) lineages are closely related to invasive infections, which confer resistance to intracellular killing and phagocytosis.¹ Aquatic environments have been described as reservoir of multidrug-resistant (MDR) bacteria, which contribute to the spread of MDR pathogens.² The emergence of MDR pathogens in several ecological spheres, including the environment, became a global threat to public health. Therefore, this study aimed to characterize an MDR-hm/hv *K. quasipneumoniae* subsp. *similipneumoniae* strain isolated from a sewage treatment plant.

Materials and Methods

In 2016, a *K. quasipneumoniae* subsp. *similipneumoniae* strain (SWT10) was recovered from a sewage treatment plant in Brasília, Distrito Federal, Brazil. The hm phenotype was determined by string test (presence of a viscous filament ≥ 5 mm after stretching using a bacteriological loop).³ The antimicrobial resistance profile was determined by the disk diffusion method and minimum inhibitory concentration (MIC) following the guidelines of the Clinical and Laboratory Standards Institute (CLSI; M100, 28th ed.) and the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCast; version 9.0, 2019).

The pattern of resistance was determined according to Magiorakos *et al.*⁴ Metal tolerance profile was performed using a range of 0.005–256 mM of copper, silver, arsenic,

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TABLE 1. GENOME ANALYSIS OF MULTIDRUG-RESISTANT AND HYPERMUCOVISCOUS/HYPERVIRULENT *KLEBSIELLA QUASIPNEUMONIAE* SUBSP. *SIMILIPNEUMONIAE* SWT10 ISOLATED FROM A SEWAGE TREATMENT PLANT

Characteristics	SWT10
Sequence type	4417
Clonal complex	1584
<i>wzi</i> allele	290
Antimicrobial resistance genes to	
β-lactams	<i>bla</i> _{OKP-B-5}
(Fluoro) quinolones	<i>oqx</i> A, <i>oqx</i> B
Tetracyclines	<i>tet</i> A
Aminoglycosides	<i>aad</i> A2
Sulfonamides	<i>sul</i> I
Trimethoprim	<i>dfr</i> A32
Macrolides	<i>ere</i> A
Fosfomycin	<i>fos</i> A
Efflux systems and regulators	<i>acr</i> A, <i>acr</i> R, <i>mar</i> A, <i>mar</i> R, <i>sox</i> R, <i>sox</i> S, <i>ram</i> A, <i>rob</i> , <i>sdi</i> A, <i>fis</i>
Heavy metal resistance genes to	
Copper	<i>pco</i> ABCDERS, <i>cop</i> AD, <i>nlp</i> E
Silver	<i>sil</i> CERS
Arsenic	<i>ars</i> CH
Chromium	<i>chr</i> A
Tellurium	<i>teh</i> AB, <i>ter</i> ABCDXFXZ
Nickel	<i>nik</i> ABCDE
Nickel/cobalt	<i>rcn</i> AB
Copper/silver	<i>cus</i> A
Magnesium/cobalt	<i>mgt</i> A
Cadmium/zinc/cobalt	<i>czc</i> A
Chromium/tellurium/selenium	<i>ruv</i> B
Magnesium/cobalt/nickel/manganese	<i>cor</i> A
Quaternary ammonium compounds	<i>sug</i> E
Virulence genes to	
Type 1 fimbriae	<i>fim</i> ABCDEFGHIK
Type 3 fimbriae	<i>mrk</i> ABCDF
Enterobactin	<i>ent</i> B
Aerobactin	<i>iut</i> A
Salmochelin	<i>iro</i> BCN
Colibactin	<i>clb</i> BL
Ferric enterobactin	<i>fep</i> CG
Ferric iron uptake	<i>kfi</i> ABC
Ferric uptake regulator	<i>fur</i>
Pullulanase secretion	<i>pul</i> BCDEFGHIJKLMNOS
<i>Escherichia coli</i> common pilus	<i>ecp</i> ABCDER
Allantoin metabolism	<i>all</i> BR
Mammalian cell entry	<i>mce</i> IG
Plasmids	IncFIB(Mar), IncHI1B, IncR, Col(IMGS31), Col440I
Prophage-related sequences (<i>n</i>)	2
Genomic islands (<i>n</i>)	66
GenBank accession number	VOIK01000000

magnesium, cobalt, nickel, manganese, cadmium, zinc, chromium, tellurium, and selenium.⁵

The genomic DNA was extracted using the PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific) and the whole genome sequencing was performed on an Illumina MiSeq platform (Illumina, Inc.), using 250 bp paired-end reads. *De novo* genome assembly was accomplished using CLC Genomics Workbench 12 (Qiagen, Denmark) and the contigs were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.3.2.⁶ Antimicrobial resistance genes, plasmid replicons, and sequence type (ST) were analyzed using ResFinder 3.2, PlasmidFinder 2.0, and MLST 2.0, respectively.^{7–9} Detection of mutations in the lipid A-Ara4N pathway (ArnA DH/FT, ArnB, ArnC, ArnT,

PmrL, PmrJ, and PhoB) and lipid A (LpxM, YciM, MrgB, PagP, PhoP, PhoQ, PmrA, PmrB, EptA, and EptB) that confer resistance to polymyxin B and colistin, respectively, was researched using the *K. quasipneumoniae* subsp. *similipneumoniae* ATCC 700603 as reference.¹⁰

The clonal complex (CC) was determined using the PHYLOViZ 2.0 software based on seven housekeeping genes (multilocus sequence typing [MLST]) of 4488 STs described so far (September 2019).¹¹ The whole-genome single nucleotide polymorphism (wgSNP) analysis was performed using the CSI Phylogeny 1.4¹² with the *K. quasipneumoniae* subsp. *similipneumoniae* ATCC 700603 as reference.¹⁰ Virulence genes, efflux systems, and regulators and capsular type were determined using the *K. pneumoniae*

FIG. 1. Minimum-spanning tree based on multilocus sequence typing of 4488 STs using the PHYLOViZ software. The CC1584 was highlighted by a dashed circle. The ST4417 was highlighted by an arrow. The numbers between circles represent the number of allelic mismatches between STs. ST, sequence type.

database.¹³ Antibacterial biocide and metal resistance genes were detected using the BacMet database.¹⁴ Phage-related sequences and genomic islands were detected using the PHASTER¹⁵ and IslandViewer 4 plus SIGI-HMM,¹⁶ respectively.

Results and Discussion

The SWT10 strain exhibited an hm phenotype (positive string test). It presented an MDR profile to cefazolin, cefuroxime, cefaclor, ceftriaxone, cefotaxime, ceftazidime, nalidixic acid, levofloxacin, ciprofloxacin, norfloxacin, lomefloxacin, ofloxacin, tetracycline, doxycycline, streptomycin, trimethoprim/sulfamethoxazole, sulfonamides, and trimethoprim and MIC 0.5 mg/L for polymyxin B and 1 mg/L for colistin, being classified as susceptible. This strain showed different MICs for metals, including copper (5 mM), silver (0.005 mM), arsenic (45 mM), magnesium (>100 mM), cobalt (1 mM), nickel (1 mM), manganese (4 mM), zinc (5 mM), cadmium (0.6 mM), chromium (2 mM), tellurium (62.5 mM), and selenium (6.25 mM).

A total of 1,279,668 (2×250 bp) paired-end reads were generated with 58× coverage. In total, 5,789 protein-coding sequences, 247 pseudogenes, 76 tRNAs, 10 ncRNAs, and 8

rRNAs were identified, with guanine-cytosine (GC) content of 57%. Resistome analysis showed antimicrobial resistance genes for β-lactams (*bla*_{OKP-B-5}), fluoroquinolones (*oqx*A and *oqx*B), tetracyclines (*tet*A), aminoglycosides (*aad*A2), sulfonamides (*sul*I), trimethoprim (*dfr*A32), macrolides (*ere*A), and fosfomycin (*fos*A). Mutations in ArnA DH/FT (T431N), ArnB (A244T), LpxM (A26T), PagP (S6A), and EptA (L11V; A154V) were detected; however, the SWT10 strain was susceptible for polymyxin B and colistin.

Five plasmid incompatibility groups were detected, including IncFIB(Mar), IncHI1B, IncR, Col(IMGS31), and Col440I. In addition, the efflux systems and regulator genes (*acr*A, *acr*R, *mar*A, *mar*R, *sox*R, *sox*S, *ram*A, *rob*, *sdi*A, and *fis*), as well as resistance genes to copper (*pco*ABCDERS, *cop*AD, and *nlp*E), silver (*sil*CERS), copper/silver (*cus*A), nickel/cobalt (*rcn*AB), arsenic (*ars*CH), magnesium/cobalt/nickel/manganese (*cor*A), nickel (*nik*ABCDE), magnesium/cobalt (*mgt*A), cadmium/zinc/cobalt (*czc*A), chromium (*chr*A), tellurium (*teh*AB and *ter*ABCDEFXZ), chromium/tellurium/selenium (*ruv*B), and quaternary ammonium compounds (*sug*E), were also detected (Table 1).

The SWT10 strain was assigned to capsular type *wzi*290-KL47. Several virulence factors were detected, such as *iut*A (aerobactin), *ent*B (enterobactin), *iro*BCN (salmochelin), *clb*BL

(colibactin), *fepCG* (ferric enterobactin), *fimABCDEFGHIK* (type 1 fimbriae), *mrkABCD* (type 3 fimbriae), *ecpABCDER* (*Escherichia coli* common pilus), *kfuABC* (ferric iron uptake), *allBR* (allantoin metabolism), *mceIG* (mammalian cell entry), *pulBCDEFGHIJKLMNOP* (pullulanase secretion), and *fur* (ferric uptake regulator). In addition, two intact phage-related sequences (*Salmonella* phage SEN5 [24.8 kb] and *Haemophilus* phage SuMu [37.5 kb]) and 66 genomic islands related to the genome evolution and microbial adaptability that ranged in size from 4,258 to 6,5905 bp (average $11,960 \pm 10,590$ bp) were detected (Table 1).

MLST analysis revealed the new allele for *phoE* gene (allele 453). The association of this new allele with other alleles belonging to the six other housekeeping genes (*gapA* [18], *infB* [22], *mdh* [18], *pgi* [16], *rpoB* [20], and *tonB* [79]) generated the new ST4417. The minimum-spanning tree showed that ST4417 grouped in CC1584 differing by a 3-loci variant (Fig. 1). The wgSNP distance from SWT10 strain to *K. quasipneumoniae* subsp. *similipneumoniae* ATCC 700603 was 0.30298. Clinical strains of *K. pneumoniae* belonging to ST1584 (CC1584) carrying extended-spectrum β -lactamase (ESBL) and *Klebsiella pneumoniae* carbapenemase (KPC) as well as virulence genes have already been reported on different continents, including in South America.^{17,18}

The STW10 strain presented the positive string test and the virulence genes that are used for the classification of hm/hv lineage.¹⁹ OKP-B-type chromosomal β -lactamases are closely related to *K. quasipneumoniae*, and OKP-B-5–producing *K. quasipneumoniae* has been commonly reported in human clinical sources, such as urine and wound.²⁰ Liver abscess and bloodstream caused by hm/hv *K. quasipneumoniae* subsp. *quasipneumoniae* were reported in North America and Europe.^{21,22} Besides, MDR ESBL-, MCR-1-, and MCR-8.2-producing *K. quasipneumoniae* have been described in animals (e.g., dog, chicken, pig, and turtle) from China, Japan, and Taiwan.^{23–26}

In Brazil, an isolate of KPC-2- and OKP-B-6–producing *K. quasipneumoniae* subsp. *similipneumoniae* was reported in nosocomial infection²⁷ and the co-occurrence of CTX-M-15 and KPC-9 in a clinical strain of MDR *K. quasipneumoniae* subsp. *similipneumoniae* was described in India.²⁸ The characteristics of hm/hv in MDR and non-MDR *K. quasipneumoniae* subsp. *similipneumoniae* have already been reported; however, not in the same strain, as described in this study (i.e., MDR-hm/hv *K. quasipneumoniae* subsp. *similipneumoniae*)^{22,29}

Conclusion

The association of antimicrobial resistance and virulence determinants represents a problem for therapy since these determinants favor the likelihood of colonization as well as the persistence of these pathogens in both patients and the environment. In summary, we report the first genome sequence of an MDR-hm/hv *K. quasipneumoniae* subsp. *similipneumoniae* recovered from the environment. This genome sequence contributes to a better understanding of MDR-hm/hv *K. quasipneumoniae* subsp. *similipneumoniae* and provides relevant data to be used in surveillance studies.

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Availability of Data

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number VOIK00000000. The version described in this article is VOIK00000000.

Disclosure Statement

No competing financial interests exist.

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References

1. Brisse, S., V. Passet, and P.A. Grimont. 2014. Description of *Klebsiella quasipneumoniae* sp. nov., isolated from human infections, with two subspecies, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* subsp. nov. and *Klebsiella quasipneumoniae* subsp. *similipneumoniae* subsp. nov., and demonstration that *Klebsiella singaporensis* is a junior heterotypic synonym of *Klebsiella variicola*. Int. J. Syst. Evol. Microbiol. 64:3146–3152.
2. Khan, F.A., B. Hellmark, R. Ehrlich, B. Söderquist, and J. Jass. 2018. Related carbapenemase-producing *Klebsiella* isolates detected in both a hospital and associated aquatic environment in Sweden. Eur. J. Clin. Microbiol. Infect. Dis. 37:2241–2251.
3. Shon, A.S., Bajwa, R.P.S., and T.A. Russo. 2013. Hypervirulent (hypermuscoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence 4:107–113.
4. Magiorakos, A.P., A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, S. Harbarth, J.F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D.L. Paterson, L.B. Rice, J. Stelling, M.J. Struelens, A. Vatopoulos, J.T. Weber, and D.L. Monnet. 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.
5. Deredjian, A., C. Colinon, E. Brothier, S. Favre-Bonté, B. Cournoyer, and S. Nazaret. 2011. Antibiotic and metal resistance among hospital and outdoor strains of *Pseudomonas aeruginosa*. Res. Microbiol. 162:689–700.
6. Tatusova, T., M. DiCuccio, A. Badretdin, V. Chetvernin, E.P. Nawrocki, L. Zaslavsky, A. Lomsadze, K.D. Pruitt, M. Borodovsky, and J. Ostell. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 44:6614–6624.
7. Zankari, E., H. Hasman, S. Cosentino, M. Vestergaard, S. Rasmussen, O. Lund, F.M. Aarestrup, and M.V. Larsen. 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67:2640–2644.
8. Larsen, M.V., S. Cosentino, S. Rasmussen, C. Friis, H. Hasman, R.L. Marvig, L. Jelsbak, T. Sicheritz-Pontén, D.W. Ussery, F.M. Aarestrup, and O. Lund. 2012. Multi-locus sequence typing of total genome sequenced bacteria. J. Clin. Microbiol. 50:1355–1361.

9. Carattoli, A., E. Zankari, A. García-Fernández, M. Voldby Larsen, O. Lund, L. Villa, F. Møller Aarestrup, and H. Hasman. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* 58:3895–3903.
10. Elliott, A.G., D. Ganesamoorthy, L. Coin, M.A. Cooper, and M.D. Cao. 2016. Complete genome sequence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain ATCC 700603. *Genome Announc.* 4:e00438-16.
11. Francisco, A.P., C. Vaz, P.T. Monteiro, J. Melo-Cristino, M. Ramirez, and J.A. Carriço. 2012. PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. *BMC Bioinformatics* 13:87.
12. Kaas, R.S., P. Leekitcharoenphon, F.M. Aarestrup, and O. Lund. 2014. Solving the problem of comparing whole bacterial genomes across different sequencing platforms. *PLoS One* 9:e104984.
13. Jolley, K.A., J.E. Bray, and M.C.J. Maiden. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res.* 3:124.
14. Pal, C., J. Bengtsson-Palme, C. Rensing, E. Kristiansson, and D.G. Larsson. 2014. BacMet: antibacterial biocide and metal resistance genes database. *Nucleic Acids Res.* 42: D737–D743.
15. Arndt, D., J. Grant, A. Marcu, T. Sajed, A. Pon, Y. Liang, D.S. Wishart. 2016. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* 44:W16–W21.
16. Bertelli, C., Laird, M. R., Williams, K. P., Simon Fraser University Research Computing Group, B.Y. Lau, G. Hoad, G.L. Winsor, and F.S.L. Brinkman. 2017. IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Res.* 45:W30–W35.
17. Yan, J.J., P.X. Zheng, M.C. Wang, S.H. Tsai, L.R. Wang, and J.J. Wu. 2015. Allocation of *Klebsiella pneumoniae* bloodstream isolates into four distinct groups by ompK36 typing in a Taiwanese University Hospital. *J. Clin. Microbiol.* 53:3256–3263.
18. Rojas, L.J., G.M. Weinstock, E. De La Cadena, L. Diaz, R. Rios, B.M. Hanson, J.S. Brown, P. Vats, D.S. Phillips, H. Nguyen, K.M. Hujer, A. Correa, M.D. Adams, F. Perez, E. Sodergren, A. Narechania, P.J. Planet, M.V. Villegas, R.A. Bonomo, and C.A. Arias. 2017. An analysis of the epidemic of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: convergence of two evolutionary mechanisms creates the “perfect storm”. *J. Infect. Dis.* 217:82–92.
19. Catalán-Nájera, J.C., U. Garza-Ramos, and H. Barrios-Camacho. 2017. Hypervirulence and hypermucoviscosity: two different but complementary *Klebsiella* spp. phenotypes? *Virulence* 8:1111–1123.
20. Long, S.W., S.E. Linson, M. Ojeda Saavedra, C. Cantu, J.J. Davis, T. Brettin, and R.J. Olsen. 2017. Whole-genome sequencing of human clinical *Klebsiella pneumoniae* isolates reveals misidentification and misunderstandings of *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*. *mSphere* 2:e00290-17.
21. Breurec, S., B. Melot, B. Hoen, V. Passet, K. Schepers, S. Bastian, and S. Brisse. 2016. Liver abscess caused by infection with community-acquired *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*. *Emerg. Infect. Dis.* 22:529.
22. Arena, F., L. Henrici De Angelis, F. Pieralli, V. Di Pilato, T. Giani, F. Torricelli, M.M. D’Andrea, and G.M. Rossolini. 2015. Draft genome sequence of the first hypermucoviscous *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* isolate from a bloodstream infection. *Genome Announc.* 3:e00952-15.
23. Li, C.F., H.L. Tang, C.S. Chiou, K.C. Tung, M.C. Lu, and Y.C. Lai. 2018. Draft genome sequence of CTX-M-type β -lactamase-producing *Klebsiella quasipneumoniae* subsp. *similipneumoniae* isolated from a Box turtle. *J. Glob. Antimicrob. Resist.* 12:235–236.
24. Yang, X., L. Liu, Z. Wang, L. Bai, and R. Li. 2019. Emergence of *mcr*-8.2-bearing *Klebsiella quasipneumoniae* of animal origin. *J. Antimicrob. Chemother.* 74:2814–2817.
25. Li, X.P., R.Y. Sun, J.Q. Song, L.X. Fang, R.M. Zhang, X.L. Lian, X.P. Liao, Y.H. Liu, J. Lin, and J. Sun. 2019. Within-host heterogeneity and flexibility of *mcr*-1 transmission in chicken gut. *Int. J. Antimicrob. Agents* S0924–S8579: 30253–30255.
26. Harada, K., T. Shimizu, Y. Mukai, K. Kuwajima, T. Sato, M. Usui, Y. Tamura, Y. Kimura, T. Miyamoto, Y. Tsuyuki, A. Ohki, and Y. Kataoka. 2016. Phenotypic and molecular characterization of antimicrobial resistance in *Klebsiella* spp. isolates from companion animals in Japan: clonal dissemination of multidrug-resistant extended-spectrum β -lactamase-producing *Klebsiella pneumoniae*. *Front. Microbiol.* 7:1021.
27. Nicolás, M.F., P.I.P. Ramos, F. Marques de Carvalho, D.R.A. Camargo, C. de Fátima Morais Alves, G. Loss de Morais, L.G.P. Almeida, R.C. Souza, L.P. Ciapina, A.C.P. Vicente, R.S. Coimbra, and A.T. Ribeiro de Vasconcelos. 2018. Comparative genomic analysis of a clinical isolate of *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, a KPC-2 and OKP-B-6 beta-lactamases producer harboring two drug-resistance plasmids from Southeast Brazil. *Front. Microbiol.* 169:220.
28. Shankar, C., S. Karunasree, A. Manesh, and B. Veerarghavan. 2019. First report of whole-genome sequence of colistin-resistant *Klebsiella quasipneumoniae* subsp. *similipneumoniae* producing KPC-9 in India. *Microb. Drug Resist.* 25:489–493.
29. Ozer, E.A., A.R. Morris, F. Krapp, C.S. Henry, K.E. Tyo, W.W. Lathem, and A.R. Hauser. 2016. Draft genome sequence of a multidrug-resistant *Klebsiella quasipneumoniae* subsp. *similipneumoniae* isolate from a clinical source. *Genome Announc.* 4:e00422-16.

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