SHORT COMMUNICATION

Whole genome sequencing reveals insights into antibiotic resistant *Klebsiella grimontii* novel sequence type ST350 isolated from a wastewater source in South Africa

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Received: July 21, 2021; **accepted:** February 23, 2022.

Klebsiella grimontii **is a recently identified species that has been implicated in clinical infections. Few or no reports on environmental *K. grimontii* exist from Africa. This study was part of a broader longitudinal research that aimed to assess the distribution, antibiotic resistance patterns, and genomics of *Enterobacterales* obtained from raw and treated wastewater and the associated river water of a wastewater treatment plant (WWTP) in KwaZulu-Natal, South Africa. We reported the genomics of an antibiotic resistant *Klebsiella grimontii* isolate obtained from the raw influent of the WWTP. Following phenotypic characterisation and antibiotic susceptibility testing, the isolate was sequenced on an Illumina MiSeq machine. Raw reads were assembled *de novo* by using SPAdes (v3.6.2) prior to bioinformatics analysis. The assembled *K. grimontii* INF139 genome was 6,369,878 bp, with 113 contiguous sequences (>200 bp) and 55.4 % GC content. The isolate was assigned a novel sequence type ST350. Genomic analysis revealed the presence of chromosomally encoded β-lactamase (*bla*$_{OXY-6-1}$) and fluoroquinolone (*oqx*B) resistance genes. Virulence factors encoding antiphagocytic, iron acquisition, and Intracellular survival properties were identified. Mobilome analysis revealed the presence of plasmid replicons (ColRNAI, FIA (pBK30683), IncFII, IncFII(Yp)) and insertion sequences (ISIX2, ISSen9, ISIS, IS1A). To our knowledge, this is the first description of antibiotic resistant *K. grimontii* from the water environment from Africa. The presence of this antibiotic resistant and potentially pathogenic isolate in the water environment is worrying as it may be disseminated into river systems used by informal settlements.**

Keywords: whole genome sequencing; antibiotic resistance; *Klebsiella grimontii*; wastewater; South Africa.

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Introduction

Klebsiella spp. are commonly implicated in human and animal infections and are widely distributed in the environment [1]. The World Health Organisation (WHO) global priority list of pathogens includes extended-spectrum β-lactamase (ESBL) Klebsiella spp [2]. Klebsiella grimontii is a recently identified species that was previously a phylogroup of K. oxytoca [3]. K.

grimontii has been implicated in clinical infections such as hemorrhagic colitis and bacteremia but may exist as a constituent of normal microflora in the intestinal tract [4]. Although several studies have reported on clinical K. grimontii [3, 5], few have investigated K. grimontii in the water environment. Surveillance of antibiotic-resistant bacteria (ARB) in the water environment remains important as wastewater treatment plants (WWTPs) and surface water bodies receive and disseminate ARB and antibiotic resistant genes (ARG) which is a public health concern, especially when communities upstream and downstream of the WWTPs use these water sources. This is common in countries where water shortages and lack of proper sewer infrastructure leads to reliance on unprocessed and recycled water [6]. The health hazards likely to result from exposure to ARB and ARG in water environments remain under investigation [7]. There is need to accelerate antibiotic resistance surveillance in the water environment to better understand the interactions and spread of ARB and ARGs in this sector. The use of genomic surveillance to understand antibiotic resistance in the environment remains limited in Africa [8]. This study was part of a longitudinal study that assessed the occurrence and dissemination of multi-drug resistant Enterobacterales of clinical and veterinary importance in a WWTP and its associated waters. We used genomics to elucidate the mechanisms of drug resistance and to reveal the location of resistance determinants and virulence genes on chromosomes or plasmids in a K. grimontii isolate obtained from the raw influent of the WWTP in KwaZulu-Natal, South Africa.

Material and methods

The study was undertaken in Pietermaritzburg, South Africa. The isolate was obtained as part of a larger study on surveillance of antibiotic resistance in a WWTP and its river water. The isolate was cultured repeatedly on Eosin Methylene Blue (EMB) lactose sucrose agar (Merck, Darmstadt, Germany) and incubated at 37˚C for 18-24 h to obtain pure colonies. Antibiotic sensitivity testing was done using the disc diffusion method against 20 antibiotics including Amikacin (AMK, 30 µg), Ampicillin (AMP, 10 µg), Azithromycin (AZM, 15 µg), Amoxicillin-clavulanic acid (AMC, 30 µg), Cefepime (FEP, 10 µg), Cefotaxime (CTX, 30 µg), Cefoxitin (FOX, 30 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CRO, 30 µg), Cephalexin (LEX, 30 µg), Ciprofloxacin (CIP, 5 µg), Chloramphenicol (CHL, 30 µg), Gentamicin (GEN, 10 µg), Imipenem (IPM, 10 µg), Meropenem (MEM, 10 µg), Nalidixic acid (NAL, 30 µg), Piperacillin-tazobactam (TZP, 110 µg), Tetracycline (TET, 30 µg), Tigecycline (TGC, 15 µg), and Trimethoprim-sulfamethoxazole (SXT, 25 µg) (Oxoid, Basingstoke, United Kingdom). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria were used to interpret results [9]. All assays included a reference strain *E. coli* ATCC 25922.

Bacterial DNA extraction, quantification, and library preparation were done as previously described [10]. An Illumina MiSeq machine (Illumina, San Diego, CA, USA) was used for whole genome sequencing (WGS). Raw reads were trimmed using Sickle v1.33 (https://github.com/najoshi/sickle) followed by *de novo* assembly using SPAdes v3.6.2. Following submission of the assembled genome to GenBank, an accession number was assigned (Bio project PRJNA609073). A novel sequence type (ST) was obtained following submission of the genome to the PubMLST *K. oxytoca*/ *michiganensis* (https://pubmlst.org/koxytoca).

The following websites were used to identify different mobile genetic elements (MGE), which included PlasmidFinder 2.1 (Plasmid replicons) (https://cge.cbs.dtu.dk/services/PlasmidFinder, ISFinder (insertion sequences) (https://isfinder.biotoul.fr), PHASTER (intact prophages) (https://phaster.ca), RAST SEEDVIEWER (integrons) (https://rast.nmpdr.org/seedviewer.cgi). VirulenceFinder 2.0 (https://cge.cbs.dtu.dk/services/VirulenceFinde) was used to find

virulence factors [10]. General Feature Format 3 (GFF3) files housed in GenBank were used to study the MGE neighboring antibiotic resistant gene (ARG) and virulence genes and to infer the probable location of the parent contigs.

The *K. grimontii* INF139 genome was compared to *K. grimontii* isolates downloaded from the Pathosystems Resource Integration Center (PATRIC) website (https://www.patricbrc.org). Phylogenetic analysis was done using the CSI Phylogeny 1.4 pipeline (https://cge.cbs.dtu.dk/services/CSIPhylogeny). *K. oxytoca* DSM29614 served as the reference strain. The generated phylogenetic tree was visualized, labelled, and amended using iTOL (https://itol.embl.de) and Figtree (http://tree.bio.ed.ac.uk/software/figtree) [10].

Results and discussion

The assembled *K. grimontii* INF139 genome was 6,369,878 bp long, with 113 contigs, 55.4 % GC content, N50 (smallest contig of the size-sorted contigs that make up at least 50% of the respective assembly) was 167,565 bp, L50 (number of contigs that make up at least 50% of the respective total assembly length) of 11 and the longest contig was 542,916 bp (Table 1). A novel ST (ST350) was assigned following submission to the PubMLST *K. oxytoca*/*michiganensis* database (https://pubmlst.org/koxytoca).

The *K. grimontii* isolate was phenotypically resistant to β-lactam antibiotics including Ampicillin (AMP), Cefoxitin (FOX), Cephalexin (LEX), and Amoxicillin-clavulanic acid (AMC) as well as Trimethoprim-sulfamethoxazole (SXT) and Tetracycline (TET) (Table 1). The resistome annotation predicted by ResFinder revealed the presence of the $bla_{\text{OXY-6-1}}$, and *oqx*B genes (Table 1). The $bla_{\text{OXY-6-1}}$ confers resistance to β-lactam antibiotics including AMC, FOX, LEX, and AMP, and is an indicator of *K. grimontii* [3, 5]. The *oqx*B gene encodes an efflux pump which confers resistance to several antibiotics including Fluoroquinolones, Chloramphenicol, and Trimethoprim [11, 12]. A phenotypic/genotypic resistance discrepancy was observed as Tetracycline resistance was not matched by any resistance determinants. The isolate was also susceptible to Ciprofloxacin, a finding similar to that reported in a study from China in which 23 *E. coli* isolates positive for the *oqx*AB gene were Ciprofloxacin sensitive [12]. The genetic environments of both the $bla_{\text{OXY-6-1}}$ and *oqx*B genes were devoid of any MGE and were chromosomally encoded.

K. gromontii harbored the virulence genes *ter*C, *fyu*A, and *tra*T. The *ter*C gene which confers resistance to tellurium was found as part of a tellurium resistance operon with 99% similarity to *K. grimontii* strain RHBSTW-00634 plasmid pRHBSTW-00634_2 (CP056683.1) in GenBank suggesting that it is transferable. The tellurite resistance operons have been reported in human pathogens and shown to mediate oxidative stress and to increase survival of particular strains in macrophages [13] The *tra*T gene encodes a protein that facilitates outer membrane protein complement resistance which inhibits phagocytosis by macrophages [14]. The virulence gene was located on a contig that had high similarity (99%) to *K. grimontii* strain SS141 plasmid_2 (CP044529.1). The *fyu*A gene encodes a siderophore receptor used to acquire iron from mammalian hosts was not associated with any MGEs. Although the *K. grimontii* INF139 isolate was environmental, it is probably a human pathogen (probability score = 0.642) as determined by PathogenFinder (https://cge.cbs.dtu.dk/services/PathogenFinder).

Phylogenetic scrutiny revealed that INF139 clustered with clinical and environmental isolates from a WWTP in the UK, suggesting it may be of clinical origins (Figure 1). Four IS families were identified but none of them were associated with ARGs or virulence factors (Table 2). No intact prophages were found. The *K. grimontii* isolate had four plasmid replicons that included the IncF type and Col plasmids.

Table 1. Genome attributes, sequence type, antibiotic resistance genes, virulence factors of *K. grimontii* (INF139) from the influent of a wastewater treatment plant (WWTP).

Identity	*Klebsiella grimontii* INF139
Source	Influent of WWTP in KwaZulu-Natal, South Africa
Antibiogram	FOX-AMP-AMC-TET-LEX-SXT
MLST*	ST350
Genome attributes	
Size	6,369,878 bp
GC content	55.4 %
N50	167,565 bp
L50	11
Number of contigs	113
Number of subsystems	602
Number of coding sequences	6,120
Number of RNAs	77
Accession number	SAMN14219492
Antibiotic resistance genes	
β-lactamase	$bla_{OXY-6-1}$
fluoroquinolone	*oqx*B
Plasmid replicons	ColRNAI, FIA (pBK30683), IncFII, IncFII(Yp)
Virulence factors	
Iron acquisition	*fyu*A
Antiphagocytosis	*tra*T
Intracellular survival	*ter*C
Insertion sequences	ISIX2, ISSen9, ISIS, IS1A
Prophages	No intact phages

*Novel sequence type; Ampicillin (AMP), Cefoxitin (FOX), Amoxicillin-clavulanic acid (AMC), Cephalexin (LEX), Tetracycline (TET), Trimethoprim-sulfamethoxazole (SXT).

Table 2. Genetic environment of resistance and virulence determinants found in *Klebsiella grimontii* INF139.

Isolate ID	Contig	ARG/ virulence gene	Genetic environment of ARG	Closest chromosome/plasmid (accession no.) aligned to contig
INF139 /11	15	*Oqx*B	No mobile genetic elements	*Klebsiella grimontii* KOX 60 chromosome (CP067433.1) - 99.9%
	5	$bla_{OXY-6-1}$	No mobile genetic elements	*Klebsiella grimontii* strain RHBSTW-00634 chromosome (CP056682.1) -100%
	2	*fyu*A	No mobile genetic elements	*Klebsiella grimontii* strain RHBSTW-00634 chromosome (CP056682.1) -100%
	28	*ter*C	*terE:terD:terC:terB:terA:terD::::::: terW*	*Klebsiella grimontii* strain RHBSTW-00634 plasmid pRHBSTW-00634_2 (CP056683.1) – 99%
	39	*tra*T	*traC:trbI:traW:traU:trbE::traF:traQ:trbB:trbF:traH:traG::traT:traD:traI:*	*Klebsiella grimontii* strain SS141 plasmid_2 (CP044529.1) – 99%

In conclusion, this is one of the first reports of antibiotic-resistant *K. grimontii* from the wastewater in Africa. The presence of this antibiotic-resistant and potentially pathogenic

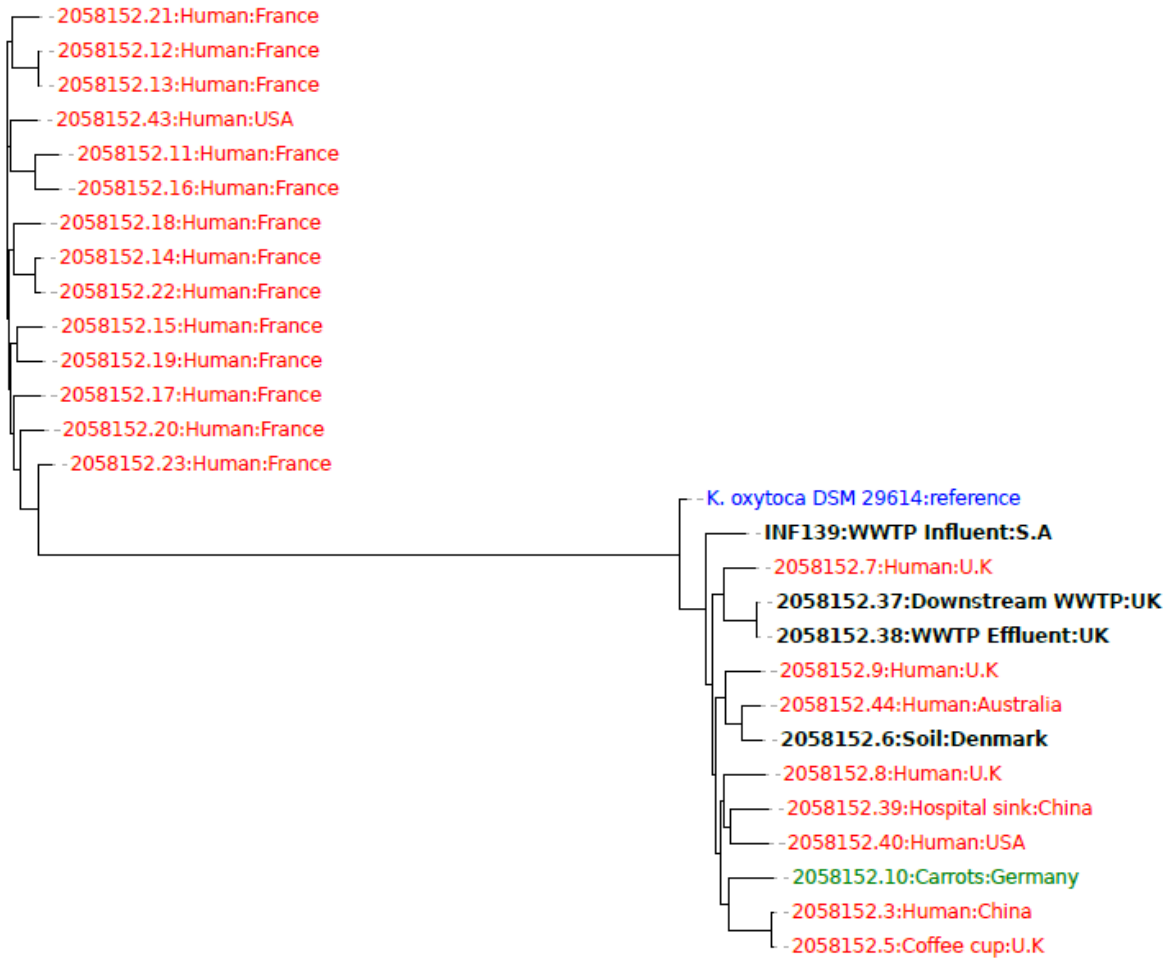

Figure 1. Phylogenetic tree of *K. grimontii* isolates from this study (INF139) and other isolates obtained from the PATRIC database.

isolate in the water environment is worrying as communities on either side of the investigated WWTP utilize the river water for household and leisure purposes.

Acknowledgements

This work is based on the research supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa (Grant no. 98342), the National Research Foundation Competitive Grant for Rated Researchers (Grant no. 106063), jointly funded by the South African Medical Research Council (SAMRC) and UK Medical Research Council Newton Fund and the SAMRC Self-Initiated Research Grant. The authors would like to thank all members of the Antimicrobial Research Unit (ARU) for their assistance.

Disclaimer

The funding source was not involved in study design, sample collection, data analysis, and interpretation. Any opinion, finding, and conclusion or recommendation expressed in this study is that of the authors, and neither the NRF nor the other funding bodies accept any liability in this regard.

Ethical consideration

Ethical approval was received from the Biomedical Research Ethics Committee (Reference: BCA444/16) of the University of KwaZulu-Natal. Permission to collect water samples was sought and granted by uMgeni Water which owns and operates the investigated WWTP.

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