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Characterization of *Klebsiella* sp. Strain 10982, a Colonizer of Humans That Contains Novel Antibiotic Resistance Alleles and Exhibits Genetic Similarities to Plant and Clinical *Klebsiella* Isolates

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A unique *Klebsiella* species strain, 10982, was cultured from a perianal swab specimen obtained from a patient in the University of Maryland Medical Center intensive care unit. *Klebsiella* sp. 10982 possesses a large IncA/C multidrug resistance plasmid encoding a novel FOX AmpC β-lactamase designated FOX-10. A novel variant of the LEN β-lactamase was also identified. Genome sequencing and bioinformatic analysis demonstrated that this isolate contains genes associated with nitrogen fixation, allantoin metabolism, and citrate fermentation. These three gene regions are typically present in either *Klebsiella pneumoniae* clinical isolates or *Klebsiella* nitrogen-fixing endophytes but usually not in the same organism. Phylogenomic analysis of *Klebsiella* sp. 10982 and sequenced *Klebsiella* genomes demonstrated that *Klebsiella* sp. 10982 is present on a branch that is located intermediate between the genomes of nitrogen-fixing endophytes and *K. pneumoniae* clinical isolates. Metabolic features identified in the genome of *Klebsiella* sp. 10982 distinguish this isolate from other *Klebsiella* clinical isolates. These features include the nitrogen fixation (*nif*) gene cluster, which is typically present in endophytic *Klebsiella* isolates and is absent from *Klebsiella* clinical isolates. Additionally, the *Klebsiella* sp. 10982 genome contains genes associated with allantoin metabolism, which have been detected primarily in *K. pneumoniae* isolates from liver abscesses. Comparative genomic analysis of *Klebsiella* sp. 10982 demonstrated that this organism has acquired genes conferring new metabolic strategies and novel antibiotic resistance alleles, both of which may enhance its ability to colonize the human body.

The *Klebsiella* genus consists of diverse organisms that are capable of colonizing and causing disease in humans and animals or existing as endophytes that colonize plants (1). *Klebsiella pneumoniae* isolates associated with human disease have been linked to pneumonia, meningitis, bacteremia, and urinary tract infections (1). Additional *Klebsiella* species capable of nitrogen fixation have been isolated from the roots of plants, where they occur in a mutualistic relationship as endophytes (2, 3). The *Klebsiella* genus originally included the species *Klebsiella planticola*, *Klebsiella terrigena*, and *Klebsiella ornithinolytica*, which have been recently described as a new genus, *Raoultella* (4). Furthermore, there are phylogenetically and phenotypically diverse *K. pneumoniae* isolates that likely represent distinct species, such as the recently described *Klebsiella variicola* (5). This highlights the fact that the nomenclature and identification of *Klebsiella* species are complex. For the purposes of this study, the *Klebsiella* species investigated are the human disease-associated *K. pneumoniae* and the nitrogen-fixing *Klebsiella* species typified by *K. variicola*.

K. pneumoniae was previously thought of predominantly as a community-acquired agent of infection but recently has become more prevalent as a nosocomial pathogen of infections such as pneumonia, meningitis, septicemia, and urinary tract infections (1, 6, 7). In recent years, *K. pneumoniae* isolates have been linked to liver abscesses; they initially were identified in Taiwan (8, 9) but subsequently have been identified globally (1, 10, 11). A number of putative virulence-associated factors have been identified among clinical isolates, including many common virulence mechanisms such as capsule production, iron acquisition, adhesion, and host resistance (1, 12). Genome sequencing of the *K. pneumoniae* liver abscess isolate NTUH-K2044 demonstrated the presence of multiple genomic insertions, in comparison with the genome of *K. pneumoniae* MGH 78578 (13). These genomic insertions contained genes associated with capsular polysaccharide production, iron transport, and allantoin metabolism (13). Interestingly, some of these genomic regions were also detected among community-acquired *K. pneumoniae* clinical isolates from cases of bacteremia, liver abscesses, and urinary tract infections (13). Furthermore, they demonstrated that these genomic regions were associated with increased mortality rates for mice, suggesting that the coding regions encode factors that may increase the virulence of the isolates containing them (13). Currently, there are few large-scale genome-sequencing efforts that focus on *Klebsiella*

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species but, as genome sequencing becomes more commonplace in clinical settings, the genomes of these types of hybrid isolates will become more prevalent and the genome space occupied by the *Klebsiella* species will become more defined.

In recent years, there has been an increase in the frequency of identified *K. pneumoniae* isolates that carry β-lactam resistance genes encoding extended-spectrum β-lactamases and carbapenemases (6, 14, 15). Several studies have isolated and sequenced plasmids conferring antibiotic resistance in *K. pneumoniae*, demonstrating the diversity of these mobile elements and the resistance genes harbored on them (16–24). Genome sequencing has demonstrated that *K. pneumoniae* can possess a large suite of antibiotic resistance mechanisms, including not only plasmid-encoded resistance genes but also a number of efflux pumps located on the chromosome (25).

In the present study, the genome sequence of *Klebsiella* sp. strain 10982 was examined with an emphasis on the genomic content of this unique isolate and the novel alleles of antimicrobial resistance genes. *Klebsiella* sp. 10982 was isolated from a patient receiving treatment for congestive heart failure in the intensive care unit (ICU) at the University of Maryland Medical Center (UMMC) (26). This isolate possesses an IncA/C multiresistance plasmid, which encodes the first described FOX-10 AmpC β-lactamase. Interestingly, *Klebsiella* sp. 10982 also has nitrogen fixation genes that are typical of *Klebsiella* endophytes but are not usually observed in clinical isolates. The *Klebsiella* sp. 10982 genome also contains genes for allantoin metabolism, which, to date, have been identified only in *K. pneumoniae* clinical isolates associated with liver abscesses (10, 13). Our findings demonstrate that this isolate is a phylogenomic and metabolic intermediate between the *Klebsiella* nitrogen-fixing endophytes and the *K. pneumoniae* clinical isolates.

MATERIALS AND METHODS

Strain isolation, biochemical identification, antimicrobial susceptibility testing, and plasmid analysis. *Klebsiella* sp. strain 10982 was isolated in February 2005 from a perianal swab culture from a patient in the University of Maryland Medical Center (UMMC) intensive care unit (ICU). Biochemical identification of this isolate was performed using the Vitek 2 and API 20E biochemical assays (bioMérieux). The antimicrobial susceptibilities of *Klebsiella* sp. 10982 were determined by identifying the MIC values using the Vitek 2 system, in accordance with CLSI guidelines (27). Plasmids conferring resistance to cefoxitin or ceftazidime were isolated from *Klebsiella* sp. 10982 by filter-mating (28) to rifampin-resistant laboratory *Escherichia coli* strain J53, and transconjugants were selected by growth on LB plates supplemented with 10 μg/ml ceftazidime (USP reference standard). Plasmids of the wild-type *Klebsiella* sp. strain 10982 and the transconjugant were detected by acid-phenol extraction (29). The plasmid bands were separated by electrophoresis on a 0.7% agarose gel at 100 V/cm for 4 h and then were stained with ethidium bromide.

PCR amplification and sequencing of relevant genome features. The FOX and LEN genes of *Klebsiella* sp. 10982 were PCR amplified for sequencing using the primers FOX_F and FOX_R and the conditions listed in Table S1 in the supplemental material. Primers SHVS-F and SHVS-R were used to sequence the LEN gene. The IncA/C replicon type was PCR amplified as described previously (30, 31) (see Table S1 in the supplemental material). Representative genes from the allantoin metabolism, citrate fermentation, and nitrogen fixation gene clusters were detected by PCR assays in five individual colonies of *Klebsiella* sp. 10982. The allantoin metabolism gene *allB*, the citrate fermentation gene *citC2*, and the nitrogen-fixation gene *nifH* were PCR amplified using primers developed in this study (see Table S1 in the supplemental material). The PCR amplicons were visualized by electrophoresis at 100 V/cm for 60 to 75 min on a 1% agarose gel containing ethidium bromide. The reaction conditions used for each primer pair are listed in Table S1 in the supplemental material. PCR amplicons obtained from a single *Klebsiella* sp. 10982 colony were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using BigDye Terminator chemistry (Applied Biosystems) on a Genetic Analyzer 3130 system (Applied Biosystems), at the University of Maryland Institute for Genome Sciences, Genome Resource Center, to confirm amplification of the target gene.

Genome sequencing and assembly. *Klebsiella* sp. 10982 was grown overnight in LB broth, and its genomic DNA was isolated using the Sigma GenElute genomic kit (Sigma-Aldrich). The genome sequence of *Klebsiella* sp. 10982 was generated using paired-end libraries with 300-bp inserts on the Illumina HiSeq 2000 system at the University of Maryland Institute for Genome Sciences, Genome Resource Center. The draft genome is 6.1 Mb, with approximately 104× sequence coverage, and was assembled by using Minimus (32) to merge contigs generated from the Velvet assembler (33) with optimal *k*-mer values determined using VelvetOptimiser v2.1.4 (see http://bioinformatics.net.au/software.velvetoptimiser.shtml) and contigs generated using the Edena v3 assembler (34). The final assembly contains 218 contigs that are ≥500 bp.

Phylogenetic analyses. The whole-genome phylogeny of select *Klebsiella* and *Enterobacter* genomes that are available in the public domain (see Table S2 in the supplemental material) was constructed as described previously (35). Briefly, the genome sequences were aligned using Mugsy (36). The homologous blocks in each genome were aligned and concatenated with the bx-python toolkit (see https://bitbucket.org/james_taylor/bx-python). A maximum-likelihood phylogeny with 100 bootstrap replicates was generated using RAxML v7.2.8 (37) and visualized using FigTree v1.3.1 (see http://tree.bio.ed.ac.uk/software/figtree).

The single-gene comparisons for the *rpoB*, LEN, and FOX genes were performed using MEGA5 (38). The *rpoB* partial nucleotide sequence phylogeny was constructed in MEGA5 (38) using the neighbor-joining method, with a Kimura 2-parameter model and 1,000 bootstrap replications. The *rpoB* phylogenetic analysis was performed using partial gene sequences for comparison with a collection of partial *rpoB* gene sequences that were previously submitted to GenBank as part of a study by Alves et al. (39). The FOX and LEN amino acid sequence phylogenies were constructed in MEGA5 (38) using the neighbor-joining method and a *p*-distance model, with 1,000 bootstrap replications.

BLAST score ratio analysis. BLAST score ratio (BSR) analysis was performed as previously described (40). Briefly, the predicted amino acid sequences of the genes of interest were compared with the draft genome of *Klebsiella* sp. 10982 and other selected genomes using TBLASTN (41). BSR values were calculated by dividing the TBLASTN (41) bit score for each protein against a single genome by the bit score obtained by TBLASTN (41) with the nucleotide sequence of the protein. The proteins were considered to be present but divergent in a genome if they had BSR values of ≥0.4 and ≤0.8, while those with BSR values of ≥0.8 were considered highly similar.

Large-scale BSR analysis (42) was used to identify the shared and unique features present in the *Klebsiella* sp. 10982 genome, in comparison with the other *Klebsiella* genomes listed in Table S2 in the supplemental material. Briefly, contigs from unfinished genomes were concatenated together and separated by a linker that adds a stop codon in all readable frames. Genes were predicted with Glimmer 3 (43), using default settings. Then the genes from all genomes were concatenated into a single file. This data set was dereplicated by clustering genes with UCLUST (44), using an identity threshold of 90% and an identity definition value of 3; a consensus representative sequence for each cluster was returned. The sequences representative of each cluster were translated and a file containing the predicted amino acid sequences was compared with each genome using TBLASTN (41). The TBLASTN (41) bit score for each feature was then divided by the maximal bit score for that feature to determine the BSR value. Using BSR analysis, a feature was considered present with high

TABLE 1 Biochemical comparisons of *Klebsiella* species[a]

Biochemical parameter	*K. pneumoniae* subsp. *pneumoniae*	*K. pneumoniae* subsp. *ozaenae*	*K. pneumoniae* subsp. *rhinoscleromatis*	*K. variicola*	*Klebsiella oxytoca*	*Raoultella planticola*	Strain 10982	*E. aerogenes*
Indole	−	−	−	−	+	v[b]	−	−
Lysine	+	v	−	v	+	+	+	+
Ornithine	−	−	−	−	−	−	−	+
Motility	−	−	−	−	−	−	−	+
Citrate	+	+	−	+	+	+	+	+
Urease	+	−	−	+	+	+	+	NK
Adonitol	+	+	+	−	NK[c]	NK	−	NK
Malonate	+	−	+	+	+	+	−	+
Voges-Proskauer	+	−	−	v	+	v	+	+
Methyl red	−	+	+	v	−	+	−	−

[a] Findings for the other *Klebsiella* spp. are from published data (1, 45) and expected Vitek 2 results.
[b] v, variable.
[c] NK, not known.

identity (BSR value, ≥0.8), present but with sequence divergence (BSR values, ≥0.4 and ≤0.8), or absent (BSR value, <0.4).

Nucleotide sequence accession numbers. The FOX-10 and LEN-26 sequences were deposited in GenBank under accession numbers JX049131 and JX124390, respectively. The *Klebsiella* sp. 10982 draft genome sequence was deposited in GenBank under accession number AKYX01000000.

RESULTS AND DISCUSSION

Biochemical and phylogenomic identification of *Klebsiella* sp. strain 10982. *Klebsiella* sp. 10982 represents a unique lineage of *Klebsiella* that has not been studied in detail or sequenced to date. Biochemical analyses using the API 20E and Vitek 2 biochemical assays identified *Klebsiella* sp. 10982 as *K. pneumoniae. Klebsiella* sp. 10982 is nonmotile, positive for lysine decarboxylase, negative for ornithine decarboxylase, and positive in the Voges-Proskauer test (Table 1), i.e., test results used to identify an organism as *Klebsiella* (1, 45). However, *Klebsiella* sp. 10982 varied from *K. pneumoniae* subsp. *pneumoniae* in the results of six diagnostic tests (adonitol, β-*N*-acetyl-glucosaminidase, β-*N*-acetyl-galactosaminidase, malonate, 5-keto-D-gluconate, and L-malate assimilation).

In addition, *Klebsiella* sp. 10982 cannot be identified to the species level based on any single housekeeping gene analysis alone, due to the lack of similarity of *Klebsiella* sp. 10982 to other characterized *Klebsiella* spp. (see Fig. S1 in the supplemental material). Phylogenetic analysis of housekeeping genes demonstrated previously that *K. pneumoniae* can be subdivided into two lineages (39, 46). The lineage of *K. pneumoniae* that contains the endophytes has recently been described as a separate species designated *K. variicola* (5). Note that some of the previously named *K. pneumoniae* isolates would be redesignated *K. variicola* under the new naming convention (for example, *K. pneumoniae* 342) (Fig. 1). Interestingly, the *rpoB* nucleotide sequence phylogeny inferred from a partial alignment of *Klebsiella rpoB* sequences previously used to subclassify *K. pneumoniae* into three phylogenetic groups (39) demonstrated that *Klebsiella* sp. 10982 falls outside the groups formed by *K. pneumoniae* and *K. variicola* (see Fig. S1 in the supplemental material). The complete *Klebsiella* sp. 10982 *rpoB* nucleotide sequence had 97% identity with the *rpoB* sequences of *K. pneumoniae*, *K. variicola*, and *Enterobacter aerogenes*, whereas BLASTN analysis of the partial *rpoB* region used in the phylogenetic analysis indicated 99% nucleotide identity with the *rpoB* of *E. aerogenes* genome sequences, demonstrating that housekeeping gene analysis cannot be used to identify *Klebsiella* sp. 10982 to the species level. Of the seven *K. pneumoniae* multilocus sequence typing (MLST) loci from *Klebsiella* sp. 10982, four of the genes (*infB*, *gapA*, *mdh*, and *pgi*), were identical to alleles in the *K. pneumoniae* database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). The other three MLST markers (*rpoB*, *phoE*, and *tonB*) from *Klebsiella* sp. 10982 had 99%, 97%, and 93% nucleotide identity, respectively, with alleles in the MLST database; therefore, strain 10982 has a unique sequence type, which was designated ST-1155.

These phenotypic and single-gene-based phylogenetic identification inconsistencies prompted us to use whole-genome sequencing to aid in the identification of this organism, which is designated *Klebsiella* sp. 10982. Whole-genome phylogenetic analysis of the *Klebsiella* sp. 10982 genome demonstrated that this isolate is on a separate phylogenetic branch, located between the groups of sequenced clinical *K. pneumoniae* and *K. variicola* endophyte genomes (Fig. 1). All of the *K. pneumoniae* clinical isolate genomes formed a single phylogenomic group. The endophyte genomes, *K. variicola* At-22 and *K. pneumoniae* 342, were in a separate lineage that also included the *Klebsiella* sp. 1_1_55 genome, which was sequenced as part of the Human Microbiome Project; however, no clinical source information is available for this isolate. The whole-genome comparison confirmed the initial findings that the *Klebsiella* sp. 10982 isolate is within the *Klebsiella* genus, but it appears to be a distinct intermediate between clinical and environmental isolates.

The genomic features of *Klebsiella* sp. 10982 were compared, using large-scale BLAST score ratio (42) analysis, with eight genomes belonging to the *K. pneumoniae* phylogroup and three genomes of the *K. variicola* phylogroup. A total of 12,224 features were predicted from the 12 *Klebsiella* genomes analyzed, which were then compared using TBLASTN analysis of the amino acid sequence of each feature in comparison with each genome. There were 4,108 features that were present with BSR values of ≥0.8 in the three *K. variicola* phylogroup genomes, the eight *K. pneumoniae* phylogroup genomes, and the *Klebsiella* sp. 10982 genome. These features represent the conserved core among these multiple species of *Klebsiella* genomes. Among these are multiple alleles of the same gene that were identified as distinct clusters when they exhibited <90% nucleotide identity, as a result of the species diversity of these genomes.

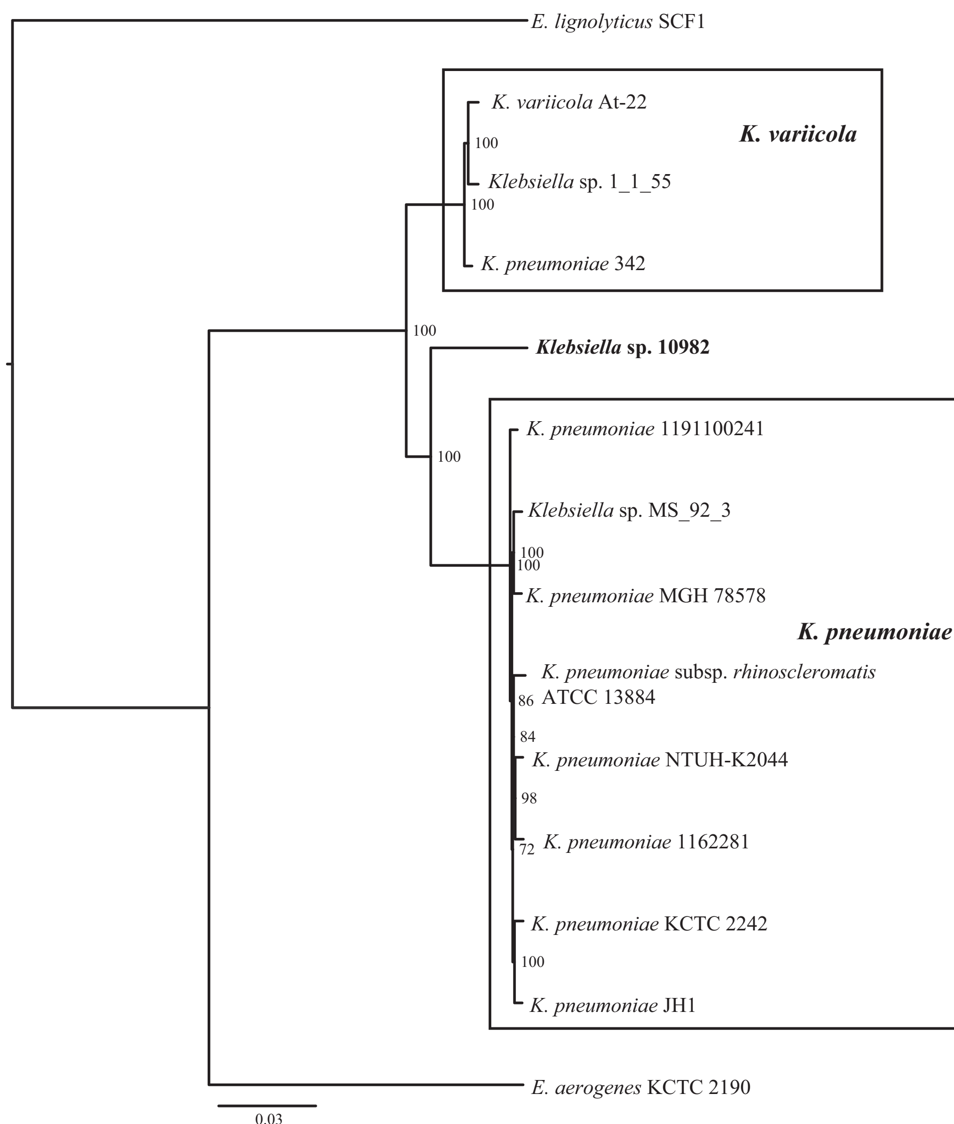

FIG 1 Whole-genome phylogeny of *Klebsiella* sp. strain 10982 relative to other *Klebsiella* isolates that have been sequenced to date. The draft or complete nucleotide sequences of the genomes were aligned using Mugsy (36). A maximum-likelihood phylogeny was constructed with 100 bootstrap replicates using RAxML v7.2.8 (37) and was visualized using FigTree v1.3.1 (see http://tree.bio.ed.ac.uk/software/figtree). Bootstrap values of ≥50 are shown. The scale bar indicates the distance of 0.03 nucleotide substitutions per site. Information regarding genomes in the phylogeny and the GenBank accession numbers is available in Table S2 in the supplemental material.

There were 136 genomic features present in *Klebsiella* sp. 10982 and all three *K. variicola* genomes that were absent from all eight of the *K. pneumoniae* genomes (see Table S3 in the supplemental material). These features represent the endophyte-like genome content of *Klebsiella* sp. 10982. Among these features were the genes for nitrogen fixation. Meanwhile, *Klebsiella* sp. 10982 shared 82 features with all eight of the *K. pneumoniae* genomes that were not present in any of the three *K. variicola* genomes (see Table S3 in the supplemental material), representing the *K. pneumoniae*-like genome content of *Klebsiella* sp. 10982. Some of these genes, as expected, encode factors that are thought to be responsible for adaptation and survival in the human host, such as a type I fimbria-like protein, putative transporter proteins, and the UmuCD DNA polymerase subunits (see Table S3 in the supplemental material). Interestingly, there were 143 features present in all *K. variicola* genomes that were also present in all *K. pneumoniae* genomes but were absent from the *Klebsiella* sp. 10982 genome, further demonstrating the unique nature of this isolate. Meanwhile, 611 features were unique to the *Klebsiella* sp. 10982 genome and were not detected in any of the other *Klebsiella* genomes analyzed (see Table S4 in the supplemental material). Among these were genes encoding an AmpC β-lactamase, plasmid-associated conjugal transfer proteins, phage-associated structural proteins, and numerous hypothetical proteins (see Table S4 in the supplemental material). In comparison, there were 288 features detected in all three *K. variicola* genomes that were not present in *Klebsiella* sp. 10982 or any of the *K. pneumoniae* genomes and 160 features detected in all eight *K. pneumoniae* genomes that were not present in any *K. variicola* genomes or *Klebsiella* sp. 10982.

Identification of metabolic and virulence-associated genes in *Klebsiella* sp. 10982. Bioinformatic detection of known metabolic and virulence-associated genes in the draft genome se-

TABLE 2 Virulence-associated and metabolic gene contents of *Klebsiella* sp. strain 10982 in comparison with other sequenced genomes

			K. variicola strain[a]			*Klebsiella* sp. strain	*K. pneumoniae* strain							
Description	Gene(s)	GenBank accession no.	342	At-22	K1_1_55	10982	NTUH-K2044	MGH 78578	1162281	JH1	MS 92-3	1191100241	ATCC 13884	KCTC 2242
Metabolic														
Nitrogen fixation	*nifQ* to *nifJ*	YP_002237547.1 to YP_002237566.1	+	+	+	+	−	−	−	−	−	−	−	−
Nitrogen assimilation from allantoin[b]	*allS* to *arc*	YP_002918199.1 to YP_002918216.1	−	−	−	+	+	−	−	−	−	−	−	−
Citrate fermentation[b]	*citB* to *citG2*	YP_001333718.1 to YP_001333728.1	+	+	+/−	+	+/−	+	−	−	+/−	+	−	+
Virulence-associated														
Iron acquisition	*iroN*, *iroB*, *iroC*, *iroD*	BAF49477.1 to BAF49480.1	−	−	−	−	+	−	−	−	+	−	+	−
Virulence-associated	*vagC*, *vagD*	BAF49474.1, BAF49475.1	−	−	−	−	+	−	−	−	+	−	+	−
Capsular polysaccharide synthesis, mucoid phenotype	*rmpA*	YP_001687850.1	−	−	−	−	+	−	−	−	−	−	+	+
Serum resistance	*magA*	BAC76772.1	−	−	−	−	+	−	−	−	−	−	−	−
Iron acquisition	*kfu*	BAH62695.1	+	+	+	+	+	+	+	+	+	+	+	+
Capsule production	*uge/wcaG*	AAP68521.1	+	+	+	+	+	+	+	+	+	+	+	+
Capsule production	*wabG*	AAX20104.1	+	+	+	+	+	+	+	+	+	+	+	+
Urease	*ureA*	ABR78861.1	+	+	+	+	+	+	+	+	+	+	+	+
Type 1 fimbrial adhesin	*fimH*	AAA25091.1	+	+	+	+	+	+	+	+	+	+	+	+

[a] +, BSR value of ≥0.8 determined by TBLASTN analysis of the amino acid sequences encoded by each gene; +/−, BSR value of ≥0.8 for one or a few of the amino acid sequences encoded by genes within this region.
[b] Also associated with virulence.

quence of *Klebsiella* sp. 10982 demonstrated that this isolate possesses genes associated only with *K. pneumoniae* clinical isolates, as well as some genes that are typically found in *K. variicola* isolates from plants (Table 2). There are far fewer sequenced *Klebsiella* plant isolate genomes, so it is difficult to make conclusive determinations about the restriction of genes to *K. variicola* isolates. *K. pneumoniae* virulence-associated genes that enhance iron acquisition (*kfu*), contribute to the mucoid phenotype (*rmpA*), or lead to the development of serum resistance (*magA*) were not detected in the genome of *Klebsiella* sp. 10982 (Table 2). Interestingly, metabolic genes detected in *Klebsiella* sp. 10982 included genes for nitrogen fixation, allantoin metabolism, and citrate fermentation (Table 2).

The *nif* gene cluster has been extensively characterized in *K. pneumoniae* (3, 47); however, some isolates that possess the *nif* gene cluster are most likely members of the *K. variicola* subgroup described above (5). *K. variicola* has been isolated from both environmental (2, 3) and clinical (5, 39) specimens; however, the genetic contents and functionality of the *nif* gene cluster in the clinical isolates have not been fully investigated. The predicted *nif* gene cluster of *Klebsiella* species contains 19 or 20 genes (47, 48) and all are present in *Klebsiella* sp. 10982 (see Table S5 in the supplemental material), suggesting that nitrogen fixation by this isolate could be possible. The *nif* genes of *Klebsiella* sp. 10982 share ≥98% nucleotide identity with those of plant *Klebsiella* isolate 342 (3). These genes were also present with similar orders and high levels of identity in the other available endophyte genome, that of *K. variicola* At-22 (2), as well as the uncharacterized *Klebsiella* isolate 1_1_55 genome (GenBank accession no. ACXA00000000). Nitrogen fixation is a bacterial characteristic more commonly identified in free-living bacteria in soils or in symbiotic associations with plants or insects (2, 3, 49). *Burkholderia vietnamiensis* is an example of a nitrogen-fixing bacterium that has been isolated from both environmental samples and human specimens (50). Recently, *B. vietnamiensis* isolates from cystic fibrosis patients have been shown to possess a deficient form of the *nif* gene cluster (50), suggesting that nitrogen fixation may play a stronger role in environmental survival than in colonizing humans. It is unclear what role, if any, the nitrogen fixation genes may have in colonization of the human gastrointestinal tract.

In select *K. pneumoniae* clinical isolates and *Klebsiella* sp. 10982, there is a 22-kb genomic island that contains genes for allantoin metabolism (see Table S5 in the supplemental material). The genes in this region of the *Klebsiella* sp. 10982 genome display 96 to 100% nucleotide identity with the allantoin metabolism genes in indel4 in the genome of the liver abscess isolate *K. pneumoniae* NTUH-K2044 (13). The allantoin metabolism genes of *Klebsiella* sp. 10982 were detected on two different contigs; however, the genes located adjacent to each end of this region in *Klebsiella* sp. 10982 are homologous to those in the *K. pneumoniae* NTUH-K2044 genome, demonstrating that this island is inserted in the same genomic site. While nearly all of the genes within the allantoin metabolism island of *Klebsiella* sp. 10982 had ≥98% nucleotide identity with those of *K. pneumoniae* NTUH-K2044, the genes on both sides of this region had less similarity to those of *K. pneumoniae* NTUH-K2044 (91 to 96% nucleotide identity). Features of the *Klebsiella* sp. 10982 genome also exhibited similarity to some genes in other indels of *K. pneumoniae* NTUH-K2044, including the type 1 fimbrial genes of indel2 and putative hydrolase-encoding genes of indel6 (data not shown). However, not all

TABLE 3 Polymorphic sites of the plasmid-encoded FOX-10 of *Klebsiella* sp. strain 10982 in comparison with other FOX sequences

Allele	GenBank accession no.	Source	Amino acid composition at polymorphic site[a]:																																						
			7	11	24	31	32	43	49	105	112	122	144	145	153	158	172	174	177	181	190	193	217	218	228	231	233	237	256	267	268	270	271	280	284	295	298	302	306	308	313
FOX-1	CAA54602	*K. pneumoniae*	F	T	R	A	A	E	M	E	Q	F	D	E	R	H	S	P	G	H	P	Q	I	A	D	V	V	V	T	A	L	T	R	Y	D	A	L	A	G	S	F
FOX-2	CAA71325	*E. coli*	L	·[b]	S	·	·	·	·	·	H	·	·	·	Q	S	·	·	·	·	·	K	M	·	·	I	·	·	V	·	·	S	A	·	·	·	V	·	·	·	·
FOX-3	CAA71947	*K. oxytoca*	·	·	S	·	T	·	I	V	·	L	·	K	Q	S	·	·	·	·	·	·	M	·	·	I	·	·	A	L	V	S	A	·	E	D	V	V	·	·	·
FOX-4	CAB89086	*E. coli*	L	M	S	·	T	A	·	·	·	·	·	·	Q	S	·	·	·	·	·	·	M	·	·	I	A	·	V	·	·	S	A	H	E	D	V	V	·	·	·
FOX-5/ FOX-6	AY007369/ AAK70221	*K. pneumoniae*	·	·	S	·	·	A	·	·	·	L	·	·	·	S	·	·	·	·	·	·	M	V	·	·	·	·	A	·	V	S	A	·	·	·	V	T	·	A	·
FOX-7	CAG28257	*Enterobacter cloacae*	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	S	A	·	·	·	·	·	·	·	·
FOX-8	ADK73994	*E. coli*	·	·	S	·	T	·	I	V	·	L	·	K	Q	S	·	·	·	·	·	·	M	·	·	I	·	·	A	L	V	S	A	·	E	D	V	V	·	·	L
FOX-9	JF896803	*K. pneumoniae*	·	·	S	V	T	A	·	·	·	·	E	·	·	S	A	T	·	Y	S	·	M	·	E	I	·	M	A	·	V	S	A	·	·	D	V	V	D	·	·
FOX-10	This study	*Klebsiella* sp. 10982	·	·	S	·	·	A	·	·	·	L	·	·	·	S	·	·	S	·	·	·	M	V	·	·	·	·	A	·	V	S	A	·	·	·	V	T	·	A	·

[a] Determined for the full-length amino acid sequence (382 amino acids).

[b] A dot indicates an amino acid sequence residue that is identical to the residue of the reference sequence in the first row.

TABLE 4 Identification of polymorphic sites in the LEN-26 sequence of *Klebsiella* sp. strain 10982 in comparison with other LEN sequences

			Amino acid composition at polymorphic site[b]:																					
Allele[a]	GenBank accession no.	Source	4	14	18	20	49	84	88	110	114	115	125	173	197	223	251	257	259	264	265	266	272	275
LEN-1	CAA28198	*K. pneumoniae*	V	T	V	Y	N	L	D	V	T	I	L	A	A	P	P	I	L	A	S	M	H	G
LEN-2	AAK69830	*K. pneumoniae*	·[c]	·	A	D	S	V	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
LEN-5	AAU25807	*K. pneumoniae*	I	·	A	·	S	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·
LEN-17	ABQ23577	*Staphylococcus epidermidis*	·	·	A	D	S	V	·	T	·	V	·	·	·	·	·	·	·	·	·	·	·	·
LEN-19	CAP12347	*K. pneumoniae*	·	·	A	·	S	·	·	T	·	V	·	·	·	·	·	·	·	·	·	·	·	·
LEN-20	CAP12348	*K. pneumoniae*	·	·	A	D	S	·	·	T	·	V	·	·	·	·	·	·	·	V	·	·	·	·
LEN-21	CAP12349	*K. pneumoniae*	·	·	A	D	S	V	V	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·
LEN-22	CAP12350	*K. pneumoniae*	·	·	A	D	S	·	·	·	·	·	·	·	P	A	·	·	·	·	·	·	·	·
LEN-23	CAP12351	*K. pneumoniae*	·	·	A	·	S	·	·	T	·	·	·	·	·	·	·	L	·	·	·	·	·	·
LEN-24	CAP12352	*K. pneumoniae*	·	N	A	D	S	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
LEN-25	ADW77562	*K. pneumoniae*	·	·	A	D	S	·	·	·	A	·	·	·	·	·	·	L	·	·	·	·	·	·
β-Lactamase	ZP_06549003	*Klebsiella* sp. 1_1_55	·	·	A	D	S	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·
β-Lactamase	YP_003439655	*K. variicola* At-22	·	·	A	D	S	·	·	·	·	·	·	·	·	·	·	L	·	·	·	·	·	·
β-Lactamase	YP_002238610	*Klebsiella* sp. 342	·	N	A	D	S	·	·	·	·	·	·	·	P	A	·	·	·	·	·	·	·	·
LEN-26[d]	AEY11240	Unknown	·	N	A	·	S	V	·	T	·	·	M[e]	M[e]	·	A[e]	A	·	M[e]	·	T[e]	I[e]	Q[e]	A[e]
LEN-26[d]	This study	*Klebsiella* sp. 10982	·	N	A	·	S	V	·	T	·	·	M[e]	M[e]	·	A[e]	A	·	M[e]	·	T[e]	I[e]	Q[e]	A[e]

[a] The sequences of 1_1_55, At-22, and 342 were identified by BLASTP analysis to have similarity to the LEN β-lactamases; however, they have not been annotated as LEN-type β-lactamases.

[b] The region displayed represents positions 1 to 277 of 286 amino acids.

[c] A dot indicates an amino acid sequence residue that is identical to the residue of the reference sequence in the first row.

[d] The LEN-26 sequences represent a previously characterized sequence with little information available regarding its origin and the sequence obtained for *Klebsiella* sp. strain 10982 in this study.

[e] Amino acid residues that differ for the LEN-26 allele versus all other LEN alleles.

genes in the indels of *K. pneumoniae* NTUH-K2044 were identified in the *Klebsiella* sp. 10982 genome. In contrast to the more widely distributed citrate fermentation gene cluster, several studies have demonstrated that the 22-kb genomic island genes for allantoin metabolism are specific to particular *K. pneumoniae* clinical isolates (10, 13). This island, in addition to six other insertion regions within the *K. pneumoniae* NTUH-K2044 genome, has been detected in isolates obtained from community-acquired bacteremia, liver abscesses, and urinary tract infections (13).

Another metabolic region of interest identified in the *Klebsiella* sp. 10982 genome is a citrate fermentation gene cluster (see Table S5 in the supplemental material). The predicted proteins for citrate fermentation were also detected in the *K. variicola* endophyte genomes and select *K. pneumoniae* clinical isolate genomes; however, they were notably absent from the genome of liver abscess isolate *K. pneumoniae* NTUH-K2044 (Table 2) (13). The genomic island encoding proteins for citrate fermentation has been identified in diverse *K. pneumoniae* isolates, and the presence of this island was shown to confer growth in artificial urine medium under anaerobic conditions (51). This island has been detected in 59% of *Klebsiella* isolates from urine samples, and it is thought to enhance the physiological ability of *Klebsiella* to thrive in the harsh conditions of urine and to cause urinary tract infections (51). However, citrate fermentation does not appear to be required by all *Klebsiella* strains to cause urinary tract infections, as 41% of *Klebsiella* isolates from urine samples did not contain this island (51). Additionally, this island is not exclusive to urinary tract-colonizing *Klebsiella* isolates, as it was also identified in approximately 64% of nonurine *Klebsiella* isolates (51).

To verify the presence of all three of these unique metabolic regions within single colonies of *Klebsiella* sp. 10982, we confirmed by PCR the presence of *allB* of the allantoin gene cluster, *citC2* of the citrate fermentation gene cluster, and *nifH* of the nitrogen fixation gene cluster. Based on the phenotype, whole-genome phylogeny, and BSR analyses, *Klebsiella* sp. 10982 appears to be a phylogenomic and phenotypic intermediate between the sequenced clinical *K. pneumoniae* isolates and the nitrogen-fixing *K. variicola* isolates.

Examination of antimicrobial resistance mechanisms in *Klebsiella* sp. 10982. The antibiotic resistance gene content of *Klebsiella* sp. 10982 further demonstrates the unique nature of this isolate. Antimicrobial susceptibility testing of *Klebsiella* sp. 10982 demonstrated that it is resistant to ceftriaxone (MIC of ≥32 μg/ml), cephalothin (MIC of ≥256 μg/ml), and ampicillin/sulbactam (MIC of ≥32 μg/ml) and sensitive to carbapenems (meropenem and ertapenem). PCR detection of β-lactamase genes demonstrated that *Klebsiella* sp. 10982 possesses a bla_{SHV}-like gene and a bla_{FOX} gene but does not contain the bla_{TEM} or $bla_{CTX\text{-}M}$ genes, which was confirmed by genome sequencing. Sequence analysis of this FOX allele identified it as a new allele that differs from the FOX-5 allele by one amino acid (Table 3; also see Fig. S2 in the supplemental material). This allele has been designated FOX-10 in the Lahey Clinic β-lactamase classification scheme (see http://www.lahey.org/Studies).

A presumptive SHV β-lactamase-encoding gene of *Klebsiella* sp. 10982 was PCR amplified using primers to detect bla_{SHV} alleles and was sequenced; however, this sequence contained 28 amino acid differences with respect to the chromosomal *Klebsiella* $bla_{SHV\text{-}1}$ allele (52) (Table 4; also see Fig. S3 in the supplemental material). This *Klebsiella* sp. 10982 bla_{SHV}-like β-lactamase sequence had 99% nucleotide identity and an identical amino acid sequence in comparison with an unpublished β-lactamase designated LEN-26 (see http://www.pasteur.fr/ip/easysite/pasteur/en/research/plates-formes-technologiques/pasteur-genopole-ile-de-france/genotyping-of-pathogens-and-public-health-pf8/beta-lactamase-enzyme-variants/beta-lactamase-enzyme-variants), demonstrating that this sequence is more closely related to a LEN β-lactamase (Table 4; also see Fig. S3 in the supplemental material).

Using plasmid incompatibility group-specific PCR, it was determined that the FOX-10-encoding gene is associated with a plasmid of the IncA/C incompatibility group. This was determined by conjugal transfer of the FOX-10-containing plasmid to rifampin-

resistant *E. coli* laboratory strain J53 with selection with ceftazidime. Isolation of plasmid DNA from the transconjugant demonstrated the mobilization of a single large supercoiled plasmid that was greater than 120 kb. These data, taken together, identify the FOX-10 gene as being present on a large mobile antibiotic resistance IncA/C plasmid, a common antibiotic resistance plasmid in *Klebsiella* species. Thus, it is possible that *Klebsiella* sp. 10982 acquired the FOX-10-encoding IncA/C multidrug-resistance plasmid from intimate association with enteric bacteria carrying the bla_{FOX-5} allele. A total of 16 IncA/C multidrug-resistance plasmids, which confer resistance to chloramphenicol, spectinomycin, sulfonamides, AmpC β-lactamases, and most recently the NDM-1 metallo-β-lactamase (20, 53–58), have been completely sequenced. The bla_{FOX-5} allele was identified previously in *E. coli* and *K. pneumoniae* isolates that harbor a large plasmid of ≥180 kb (59, 60), but none of these plasmids has been sequenced yet. Further characterization of the gene contents and diversity of bacterial hosts that have acquired the FOX IncA/C multidrug-resistance plasmid is under way.

Conclusions. The current study describes the phenotypic and genomic characterization of *Klebsiella* sp. 10982, which contains two novel β-lactamase alleles, FOX-10 and LEN-26. It is unclear whether *Klebsiella* sp. 10982 contributed to the observed clinical presentation, as it was obtained from a perianal swab from a patient being treated in the ICU for congestive heart failure, and no other samples obtained from this patient that were positive for an extended-spectrum β-lactamase producer also contained a *Klebsiella* isolate. Further investigation is necessary to determine whether this intermediate generalist *Klebsiella* isolate is capable of causing a clinical infection or is able to colonize the human gastrointestinal tract. In addition, it remains to be determined what role, if any, harboring the genes for nitrogen fixation, allantoin metabolism, citrate fermentation, and plasmid-encoded antibiotic resistance would have in its ability to colonize and to persist in the human body.

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