

Genome misclassification of *Klebsiella variicola* and *Klebsiella quasipneumoniae* isolated from plants, animals and humans

Esperanza Martínez-Romero, PhD,⁽¹⁾ Nadia Rodríguez-Medina, M in C,⁽²⁾ Marilu Beltrán-Rojel, Biol,⁽²⁾ Jesús Silva-Sánchez, PhD,⁽²⁾ Humberto Barrios-Camacho, PhD,⁽²⁾ Ernesto Pérez-Rueda, PhD,^(3,4) Ulises Garza-Ramos, PhD.⁽²⁾

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Abstract

Objective. Due to the fact that *K. variicola*, *K. quasipneumoniae* and *K. pneumoniae* are closely related bacterial species, misclassification can occur due to mistakes either in normal biochemical tests or during submission to public databases. The objective of this work was to identify *K. variicola* and *K. quasipneumoniae* genomes misclassified in GenBank database. **Materials and methods.** Both *rpoB* phylogenies and average nucleotide identity (ANI) were used to identify a significant number of misclassified *Klebsiella* spp. genomes. **Results.** Here we report an update of *K. variicola* and *K. Quasipneumoniae* genomes correctly classified and a list of isolated genomes obtained from humans, plants, animals and insects, described originally as *K. pneumoniae* or *K. variicola*, but known now to be misclassified. **Conclusions.** This work contributes to recognize the extensive presence of *K. variicola* and *K. quasipneumoniae* isolates in diverse sites and samples.

Keywords: bacterial infections; Gram-negative bacterial infections; Enterobacteriaceae infections; nitrogen fixation

Resumen

Objetivo. Identificar genomas mal clasificados de *K. variicola*, y *K. quasipneumoniae* en la base de datos del GenBank. **Material y métodos.** En el presente estudio se usaron tanto análisis filogenéticos usando *rpoB* como la identidad media de nucleótidos (ANI, por sus siglas en inglés) para identificar un número significativo de genomas del género *Klebsiella*. **Resultados.** Se reportó una actualización de genomas de *K. variicola* y *K. quasipneumoniae* correctamente clasificados y una lista de aquellos aislamientos obtenidos de seres humanos, plantas, animales e insectos, descritos originalmente como *K. pneumoniae* o *K. variicola* pero ahora se conoce que están mal clasificados. **Conclusiones.** Este trabajo contribuye a la presencia extensiva de aislamientos de *K. variicola* y *K. quasipneumoniae* en diversos sitios y muestras.

Palabras clave: infecciones bacterianas; infecciones por bacterias gramnegativas; fijación de nitrógeno

(1) Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México. Cuernavaca, Morelos, México.

(2) Grupo de Resistencia Bacteriana, Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública. Cuernavaca, Morelos, México.

(3) Departamento de Ingeniería Celular y Biotecnología, Instituto de Biotecnología, Universidad Nacional Autónoma de México. México.

(4) Instituto de Investigaciones en Matemáticas Aplicadas y en Sistemas, Universidad Nacional Autónoma de México. Mérida, Yucatán, México.

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Corresponding author: Dr. Ulises Garza Ramos. Departamento de Diagnóstico Epidemiológico, Instituto Nacional de Salud Pública. Av. Universidad 655 col. Santa María Ahuacatlán. 62100, Cuernavaca, Morelos, México.

E-mail: ulises.garza@insp.mx

A recent taxonomic description of the genus *Klebsiella* spp. includes three taxonomic subspecies; *K. pneumoniae* subsp. *pneumoniae* (KpI), *K. pneumoniae* subsp. *rhinoscleromatis* and *K. pneumoniae* subsp. *ozaenae* and our novel species; *Klebsiella variicola* (KpIII), *K. singaporensis*, *K. michiganensis* and *K. quasipneumoniae* (KpII), which has two subspecies: *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* (KpII-A) and *K. quasipneumoniae* subsp. *similipneumoniae* (KpII-B).¹ For these bacterial species novel genome sequences have been described from the strains 07A044, 18A069¹ and FIHV2014.² The misclassification of *K. variicola* and *Klebsiella* spp. genomes was first identified by whole-genome and *rpoB* phylogenies.^{3,4} Subsequently by *K. quasipneumoniae* and other *K. variicola* genomes the misclassification was evidenced by Average Nucleotide Identity (ANI).⁵ The aim of this work was detect misclassified *K. variicola* and *K. quasipneumoniae* genomes. We used an *rpoB* gene phylogenetic analysis and the results were validated by ANI. We identified the isolates misclassified previously described⁵ and identified new isolates that corresponded to *K. variicola* and *K. quasipneumoniae* that were deposited as *K. pneumoniae*. Likewise, we identified one *K. variicola* genome misclassified that corresponds to *K. quasipneumoniae* subsp. *similipneumoniae*.

Materials and methods

Identification of the *K. variicola* and *K. quasipneumoniae* genomes

The complete nucleotide sequence of *rpoB* (KVR801 v1_470024) gene from *K. variicola* 801 (CDMV00000000) was downloaded from the European Nucleotide Archive database.⁶ The *rpoB* sequence was analyzed by the BLASTn option (with default values) against 419 complete and draft *Klebsiella* spp. (tax id: 570) genomes deposited in GenBank database (last analysis 13/07/2015).⁷ The *rpoB* genes from *Escherichia coli* K-12-MG1655, *Salmonella enterica* Ty21a, *Klebsiella oxytoca* KCTC1686, *K. quasipneumoniae* subsp. *quasipneumoniae* 18A069 (KpII-A), *K. quasipneumoniae* subsp. *quasipneumoniae* 01A030 (KpII-A) and *K. quasipneumoniae* subsp. *similipneumoniae* 07A044 (KpII-B) genomes¹ were included in the phylogenetic analysis. Subsequently, the *rpoB* nucleotide sequences of *K. pneumoniae* genomes with 100% nucleotide identity were filtered out to exclude a possible bias. All strain genomes identified as *K. variicola* and *K. quasipneumoniae* were included. Finally, a phylogenetic reconstruction was performed using the maximum-likelihood method with a Tamura-Nei-parameter model and 1 000 bootstrap replications (Mega v6.06).⁸

The ANI was determined using the Average Nucleotide Identity Calculator⁹ the default parameters. In total, thirty-one *K. variicola*, nine *K. quasipneumoniae* and two *K. pneumoniae* genomes were downloaded from GenBank and analyzed (table I). *K. variicola* At-22 (CP001891), *K. quasipneumoniae* subsp. *quasipneumoniae* 18A069 (CBZM000000000), *K. quasipneumoniae* subsp. *similipneumoniae* 07A044 (CBZR000000000) and *K. pneumoniae* MGH78578 (CP000647) were used as reference genomes of the respective bacterial species.

Results and discussion

Klebsiella spp. genomes that corresponded to *K. variicola* and *K. quasipneumoniae*

Both *K. variicola* as *K. pneumoniae* and *K. quasipneumoniae* isolates exhibit very similar biochemical features,¹ and to date there are no reported biochemical tests for their appropriate differentiation.^{3,4,10,11} The phylogenetic analysis of the *rpoB* gene is more adequate than the 16S rRNA for *Klebsiella* spp. differentiation.¹² Likewise, *K. variicola* DX120E was identified both by 16S rRNA as *rpoB* genes, however, a better resolution was obtained with *rpoB* gene analysis.¹³ In addition, *K. variicola* described in animals and plants was also included in the *rpoB* phylogenetic analysis.³ Previously concatenated housekeeping genes were proposed to differentiate of *Klebsiella* species.^{1,3} With this criterium *K. pneumoniae* 342 from maize,¹⁴ and *K. pneumoniae* KP5-1 from insect¹⁵ were found to be misclassified as *K. pneumoniae*.³ Actually the new *K. variicola* and *K. pneumoniae* isolates may now be properly identified by PCR. The first multiplex PCR, M-PCR-1 based on unique genes from each species would allow the differentiation in clinical laboratories of *K. variicola* and *K. pneumoniae* bacterial species.³ In the present study, the nucleotide BLAST analyses of *rpoB* gene sequence allowed the analysis of 419 *Klebsiella* spp. Genomes. All those strains with *rpoB* genes with 100% nucleotide identity to *rpoB* of *K. pneumoniae* MGH78578 were not further considered, as they had a correct taxonomic affiliation. 144 non-redundant DNA sequences were used for a phylogenetic analysis, showing seventeen putative *K. pneumoniae* strains corresponding to *K. variicola* bacterial species (figure 1). Their reported identification was *K. pneumoniae* MGH20, MGH40, MGH68, MGH76, MGH80, MGH92, MGH114, UCI18, BIDMC61, BIDMC88, BIDMC90, 223/14, KTE92, UCICRE10, B1, and CH4 and *Klebsiella* spp. 1.1.55. The *Klebsiella* spp. 1.1.55 clinical isolate was previously identified as a possible *K. variicola*.⁴ In addition, *K. pneumoniae* UCICRE14 corresponded to *K. quasipneumoniae* subsp.

Table I
AVERAGE NUCLEOTIDE IDENTITY (ANI) BETWEEN BACTERIAL GENOMES FROM ALL ISOLATES AS QUERIES, AND *K. VARIICOLA*, *K. PNEUMONIAE* AND *K. QUASIPNEUMONIAE* REFERENCE STRAIN GENOMES

	Average nucleotide identity (%)		
	<i>K. variicola</i> At-22	<i>K. pneumoniae</i> MGH78578	<i>K. quasipneumoniae</i> 18A069
<i>K. variicola</i>			
At-22	100.00	94.07	92.90
223/14	99.18	94.14	92.98
BI	99.17	94.08	92.91
CH4	99.18	94.10	92.90
MGH20	99.18	94.08	92.94
MGH40	99.15	94.13	92.95
UCICRE10	99.19	94.14	92.91
UCI18	99.19	94.15	92.94
BIDMC61	99.16	94.05	92.95
MGH68	99.15	94.12	92.89
MGH76	99.19	94.15	93.03
MGH80	99.18	94.09	93.03
BIDMC88	99.14	94.12	92.94
BIDMC90	99.10	94.14	93.02
MGH114	99.19	94.12	92.93
MGH92	99.12	94.09	92.93
I.I.55	99.13	94.12	92.94
KTE92	99.13	94.13	92.95
342	99.08	94.13	92.93
KP5-I	99.13	94.07	92.89
BZ19	99.17	94.13	92.92
DX120E	99.19	94.13	92.91
DSM15968	99.13	94.13	92.90
CAG:634	99.17	94.01	92.90
801	99.16	94.09	92.95
8917	99.19	94.14	92.91
06-268	99.17	94.08	92.92
3	99.21	94.07	92.88
4880	99.18	94.02	92.92
6A2	99.21	94.03	92.93
T29A	99.16	94.08	92.93
<i>K. quasipneumoniae</i>			
18A069*	92.90	93.27	100.00
01A030*	92.84	93.10	99.15
07A044‡	92.93	93.31	100.00§
UCICRE14*	92.88	93.23	99.22
12-3578‡	92.90	93.30	99.22§
ATCC 700603‡	92.92	93.31	99.32§
MGH44‡	92.91	93.39	99.06§
HKUOPLA‡	92.96	93.40	99.20§
HKUOPLC‡	92.95	93.40	99.19§
<i>K. pneumoniae</i>			
MGH78578	94.07	100.00	93.27
NTUH-K2044	94.10	99.21	93.28

* *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*

‡ *Klebsiella quasipneumoniae* subsp. *similipneumoniae*

§ *K. quasipneumoniae* subsp. *similipneumoniae* 07A044 used as reference genome in ANI calculation

quasipneumoniae (KpII-A), whereas the *K. pneumoniae* 12-3578, ATCC 700603, MGH44 and HKUOPLC and *K. variicola* HKUOPLA corresponded to *K. quasipneumoniae* subsp. *similipneumoniae* (KpII-B) (figure 1).

Validation by average nucleotide identity analysis

The results obtained by phylogenetic analysis of *rpoB* gene were confirmed by determining the ANI value (table I). *K. variicola* At-22, *K. quasipneumoniae* 18A069, *K. quasipneumoniae* subsp. *similipneumoniae* 07A044 and *K. pneumoniae* MGH78578 were used as reference genomes in the ANI platform. This platform may use draft or complete genomes.⁷ When strains from the same subspecies were compared, ANI was >99%. Among the *K. variicola* genomes, an ANI value of >99.08 was obtained; similarly, an ANI value of >99.15% for the *K. quasipneumoniae* genomes was obtained. For the *K. pneumoniae* and *Klebsiella* spp. genomes that were identified as *K. variicola* in the present study, ANI values were <94.01% compared with those of *K. pneumoniae* MGH-78578. In contrast, an ANI value of >98.89% was obtained when *K. variicola* isolates were compared to *K. variicola* At-22 (table I). Previously, Chen and colleagues⁵ detected nine misclassified *K. variicola* and four *K. quasipneumoniae* genomes. This update of *Klebsiella* genomes highlight that not only *K. pneumoniae* are misclassified, but also, *K. variicola*.

Not only *K. pneumoniae* and *K. variicola* but also *K. quasipneumoniae* isolates are not differentiated by biochemical methods. To address this problem, Brisse and colleagues¹ proposed a phylogenetic analysis based on five protein-coding genes (*rpoB*, *fusA*, *gapA*, *gyrA*, and *leuS*) that clearly differentiates *K. variicola* and *K. quasipneumoniae* from *K. pneumoniae* bacterial species. Nevertheless, we previously showed that the *rpoB* nucleotide partial gene sequence (501 bases) also supports the differentiation of *K. variicola* and *K. pneumoniae*.³ This work confirms the correct taxonomic affiliation using the complete *rpoB* gene sequence.

General characteristics of *K. variicola* and *K. quasipneumoniae* strains and genomes

Misclassified *K. pneumoniae* and *K. variicola* genomes, now know to corresponded to *K. variicola* or *K. quasipneumoniae*, were submitted to GenBank in 2009. Other misclassified strains, such as *Klebsiella* spp. 1.1.55, and *K. pneumoniae* 342 genomes were deposited in 2008 (table II). The latter was reported to correspond to *K. variicola* in subsequent studies.^{1,3,16} Genome submissions were mainly to USA, Mexico and Malaysia, with the USA

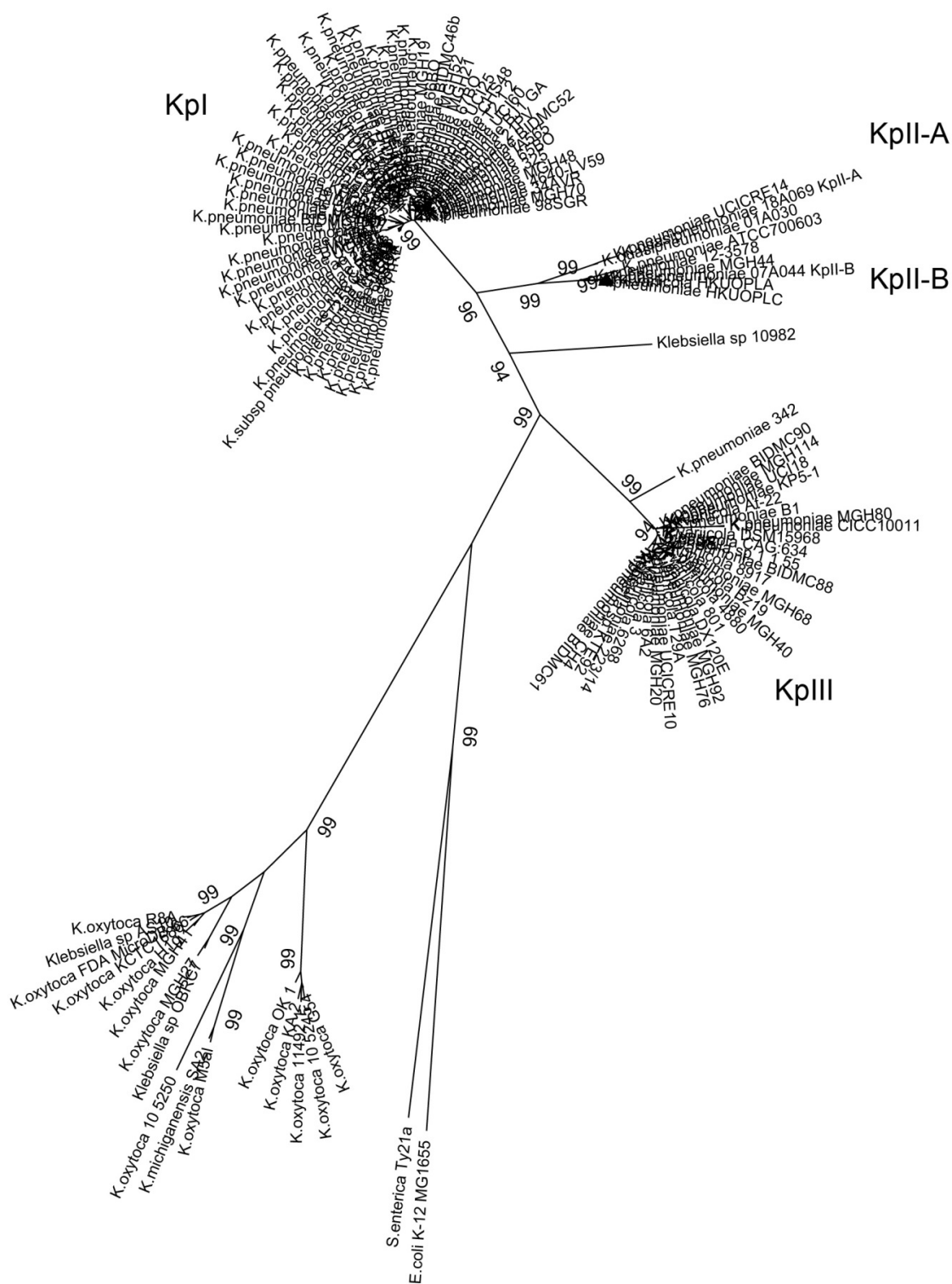


FIGURE 1. PHYLOGENETIC ANALYSIS OF THE *rpoB* GENE OF THE 144 NON-REDUNDANT *K. VARIICOLA*, *K. QUASIPNEUMONIAE* AND *K. PNEUMONIAE* GENOMES. **Kpl, *K. PNEUMONIAE*; **KpII-A**, *K. QUASIPNEUMONIAE* SUBSP. *QUASIPNEUMONIAE*; **KpII-B**, *K. QUASIPNEUMONIAE* SUBSP. *SIMILIPNEUMONIAE* AND **KpIII**, *K. VARIICOLA***

Table II
CHARACTERISTICS OF *K. VARICOLA*, *K. QUASIPNEUMONIAE* AND *K. PNEUMONIAE* GENOMES CONSIDERED IN THE STUDY

GenBank submission specie	Bacterial specie	Isolate	Origin of isolates	Submitted on public data base	Country	Sequencing method	Contigs	N50 (coverage)	Genome size (total)	CDS or proteins number data base	Accession number data base	PUBMED (PMID)
<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	NTUH-K2044	Human (blood)	06-Jan-2004	China	Sanger shotgun	1	5 248 520	5 248 520	5 270	AP006725.1	19447910
<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	MGH-78578	Human	06-Sep-2006	USA	Sanger shotgun	1	5 315 120	5 315 120	5 244	CP006647.1	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	2231/14	Human (pus)	07-Oct-2014	Malaysia	PacBio	13	5 584 553 (75X)	6 165 397	5 797	JRTV000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	BI	Plant (bitter gourd)	29-Oct-2014	Malaysia	Illumina HiSeq	90	152 679 (63X)	5 484 200	5 212	JSWX000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	CH4	Plant (chili)	29-Oct-2014	Malaysia	Illumina HiSeq	95	291 443 (74X)	5 480 893	5 223	JYKA000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	MGH20	Human (respiratory)	06-Sep-2013	USA	Illumina HiSeq 2000	25	665 487 (145X)	5 651 060	5 237	AYJK000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	MGH40	Missing (urine)	06-Sep-2013	USA	Illumina	14	858 351 (143X)	5 376 506	4 978	AYIX000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	UCICRE10	missing	06-Sep-2013	USA	Illumina HiSeq 2000	12	1 342 103 (135X)	5 376 506	4 959	AYIF000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	UCI18	Human (urine)	10-Jan-2014	USA	Illumina	18	540 261 (136X)	6 035 862	5 643	JCML000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	BIDMC61	Human (urine)	25-Apr-2014	USA	Illumina	24	493 486 (135X)	5 580 642	5 170	JMWB000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	MGH68	Human (urine)	25-Apr-2014	USA	Illumina HiSeq 2000	25	398 006 (135X)	5 521 224	5 124	JMZD000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	MGH76	Human (bile)	25-Apr-2014	USA	Illumina	400	57 253 (46X)	5 960 376	5 327	JMZK000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	MGH80	Human (urine)	25-Apr-2014	USA	Illumina	36	341 150 (139X)	5 775 155	5 394	JMZM000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	BIDMC88	Human (missing)	10-Jun-2015	USA	Illumina HiSeq 2000	30	1 342 103 (135X)	5 376 506	5 399	LFBA000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	BIDMC90	Human (missing)	10-Jun-2015	USA	Illumina	48	445 357 (132X)	6 478 359	6 153	LFAD000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	MGH114	Human	10-Jun-2015	USA	Illumina	96	293 076 (23X)	6 023 346	5 644	LFAP000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	MGH92	Human (missing)	10-Jun-2015	USA	Illumina	98	130 603 (55X)	5 642 554	5 199	LFBC000000000	Unpublished
<i>Klebsiella sp.</i>	<i>K. varicola</i>	I.1.55	Human (missing)	08-Oct-2009	USA	454 GS-FLX	96	110 877 (20X)	5 459 739	5 010	ACXA000000000	Unpublished
<i>Klebsiella sp.</i>	<i>K. varicola</i>	KTE92	Human (missing)	22-Feb-2013	USA	Illumina	34	344 789 (129X)	5 471 343	5 043	ASQN000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. varicola</i>	342	Plant (maize stems)	05-Mar-2008	USA	Sanger shotgun	1	5 641 239 (8X)	5 641 239	5 813	CP000964.1	18654632
<i>K. pneumoniae</i>	<i>K. varicola</i>	KP5-1	Insect (<i>Nezara viridula</i>)	04-Jun-2014	USA	454 GS-Junior	1	5 365 144 (16X)	5 365 144	5 198	CP008700.1	25146146
<i>K. varicola</i>	<i>K. varicola</i>	BZ19	Human (Faeces)	21-Aug-2014	Brazil	454	208	55 792 (14X)	5 543 565	5 003	JDWA000000000	25135672
<i>K. varicola</i>	<i>K. varicola</i>	DX120E	Plant (banana roots)	09-Sep-2014	China	PacBio	1	5 501 013 (96X)	5 501 013	5 172	CP009274	Unpublished
<i>K. varicola</i>	<i>K. varicola</i>	DSM15968	Plant (banana roots)	13-Jan-2015	China	PacBio	1	5 521 203 (185X)	5 521 203	4 979	CP010523	Unpublished
<i>K. varicola</i>	<i>K. varicola</i>	CAG:634	Human (gut)	28-Nov-2012	Denmark	Not founded	464	19 584	5 182 156	4 879	CBA000000000	25886267
<i>K. varicola</i>	<i>K. varicola</i>	801	Human (blood)	15-Jan-2015	Mexico	454 GS-FLX Titanium	91	131 409 (15X)	5 790 474	5 620	CDMV000000000	25886267
<i>K. varicola</i>	<i>K. varicola</i>	06-268	Human (sputum)	18-Feb-2015	Mexico	454 GS-FLX Titanium	103	257 189 (20X)	5 686 491	5 621	CEGG000000000	25888850
<i>K. varicola</i>	<i>K. varicola</i>	3	Human (abscess)	24-Jul-2015	Mexico	454 GS-FLX Plus	19	407 879 (30X)	5 431 857	5 278	CXOZ000000000	This work
<i>K. varicola</i>	<i>K. varicola</i>	4880	Plant (maize shoots)	24-Jul-2015	Mexico	454 GS-FLX Plus	24	374 829 (25X)	5 512 858	5 387	CXOY000000000	26358599
<i>K. varicola</i>	<i>K. varicola</i>	6A2	Human (blood)	24-Jul-2015	Mexico	454 GS-FLX Titanium	88	220 017 (19X)	5 415 715	5 258	CXPB000000000	This work
<i>K. varicola</i>	<i>K. varicola</i>	T29A	Plant (sugar cane stem)	24-Jul-2015	Mexico	454 GS-FLX Titanium	206	168 242 (15X)	5 925 914	5 906	CXPC000000000	26358599
<i>K. varicola</i>	<i>K. varicola</i>	At-22	Insect (lungus matrix)	26-Jan-2010	USA	454 GS-FLX Titanium	126	213 313 (18X)	5 865 668	5 751	CXPA000000000	26358599
<i>K. quasipneumoniae</i> *	<i>K. quasipneumoniae</i>	01A030	Human (blood)	16-May-2014	Austria	454 GS-FLX	1	5 458 505	5 458 505	5 309	CP001891.1	19965433
<i>K. quasipneumoniae</i> *	<i>K. quasipneumoniae</i>	18A069	Human	08-Jan-2014	Spain	Illumina	65	151 149	5 465 736	5 287	CCDF000000000	Unpublished
<i>K. quasipneumoniae</i> †	<i>K. quasipneumoniae</i>	07A044	Human	08-Jan-2014	Germany	Illumina	476	24 320	5 387 733	5 007	CBZM000000000	24958762
<i>K. pneumoniae</i>	<i>K. quasipneumoniae</i> *	UCICRE14	missing	06-Sep-2013	USA	Illumina	46	257 097	5 109 717	4 927	CBZR000000000	24958762
<i>K. pneumoniae</i>	<i>K. quasipneumoniae</i> †	12-3578	Human (blood)	15-Apr-2013	China	Illumina HiSeq	24	(140X)	5 517 176	5 054	AYIC000000000	Unpublished
<i>K. pneumoniae</i>	<i>K. quasipneumoniae</i> †	ATCC 700603	Human (blood)	15-Jan-2013	USA	Illumina HiSeq	183	136 195 (62X)	5 742 061	0	AOQO000000000	23723407
<i>K. pneumoniae</i>	<i>K. quasipneumoniae</i> †	MGH44	missing (respiratory)	06-Sep-2013	USA	Illumina	103	226 688 (800X)	5 461 663	5 231	AYIV000000000	Unpublished
<i>K. varicola</i>	<i>K. quasipneumoniae</i> †	HKUOPLA	Panda (feces)	31-Jul-2015	China	Illumina MiSeq	11	922 744 (273X)	5 240 541	4 832	AYIV000000000	26472841
<i>K. pneumoniae</i>	<i>K. quasipneumoniae</i> †	HKUOPLC	Panda (feces)	04-Aug-2015	China	Illumina MiSeq	1	5 062 551 (61X)	5 090 052	4 757	CP012252	Unpublished

**Klebsiella quasipneumoniae* subsp. *quasipneumoniae*

†*Klebsiella quasipneumoniae* subsp. *similipneumoniae*

as the major contributor (table II). These isolates were obtained from many different sources, such as humans (from urine, pus bile and other organic tissues), giant pandas (feces), different sites of plants, and from diverse insect species (table II). The majority of bacterial genomes were submitted as drafts with 12 to 400 contigs, using mostly Illumina technology. Five *K. variicola* genomes were completely sequenced using the Sanger shotgun, PacBio and 454 technologies (completed by Sanger sequencing of gaps), and the draft genomes contained from 24 to 464 contigs.

The *K. quasipneumoniae* genomes were submitted in 2013 and 2015 from different countries, including Austria, China, Germany, Spain and USA. All of these genomes were isolated from humans and were sequenced using the Illumina platform, with contigs from 1 to 476 and genome sizes >5 Mb and a content of CDS or proteins of 4 757 to 5 287 (table II).

Final considerations

It is a fact that *Klebsiella* spp. isolates have been misclassified. Several isolates of both *K. variicola* and *K. quasipneumoniae* from various sources were identified as *K. pneumoniae*. We report here, on a genomic basis, on misclassified *K. pneumoniae*, *K. quasipneumoniae* and *K. variicola* isolates from humans, animals, plants and insects. Nevertheless, the recent use of different methods such as PCR, phylogenetic analysis of *rpoB* gene and ANI for the proper differentiation of *K. variicola* and *K. quasipneumoniae* from *K. pneumoniae* will help the adequate classification of *Klebsiella* species. This work contributes to recognizing the extensive presence of *K. variicola* and *K. quasipneumoniae* in diverse sites and samples. The correct identification of *K. variicola* in the hospital setting could contribute to a more appropriate medical treatment.¹⁷ Notably, some *K. variicola*^{18,19} and *K. quasipneumoniae*,²⁰ respectively, have acquired carbapenem resistance and virulence factors.

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Referencias

1. Brisse S, Passet V, Grimont PA. 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* 2014;64(Pt 9):3146-3152. <https://doi.org/10.1099/ijs.0.062737-0>
2. Arena F, Henrici De Angelis L, Pieralli F, Di Pilato V, Giani T, Torricelli F, et al. Draft Genome Sequence of the First Hypermucoviscous *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* Isolate from a Bloodstream Infection. *Genome Announc* 2015;3(5):pii: e00952-15. <https://doi.org/10.1128/genomeA.00952-15>
3. Garza-Ramos U, Silva-Sanchez J, Martínez-Romero E, Tinoco P, Pina-Gonzales M, Barrios H, et al. Development of a multiplex-PCR probe system for the proper identification of *Klebsiella variicola*. *BMC Microbiol* 2015;15:64. <https://doi.org/10.1186/s12866-015-0396-6>
4. Hazen TH, Zhao L, Sahl JW, Robinson G, Harris AD, Rasko DA, et al. 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. *Antimicrob Agents Chemother* 2014;58(4):1879-1888. <https://doi.org/10.1128/AAC.01605-13>
5. Chen M, Li Y, Li S, Tang L, Zheng J, An Q. Genomic identification of nitrogen-fixing *Klebsiella variicola*, *K. pneumoniae* and *K. quasipneumoniae*. *J Basic Microbiol* 2016;56(1):78-84. <https://doi.org/10.1002/jobm.201500415>
6. European Nucleotide Archive [cited 2016 Jan]. Available from: <http://www.ebi.ac.uk/ena>
7. Basic Local Alignment Search Tool (BLAST) [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information [cited 2016 Jan]. Available from: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
8. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28(10):2731-2739. <https://doi.org/10.1093/molbev/msr121>
9. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57(Pt 1):81-91 [cited 2016 January]. Available from: <http://enve-omics.ce.gatech.edu/ani/>
10. Alves MS, Dias RC, de Castro AC, Riley LW, Moreira BM. Identification of clinical isolates of indole-positive and indole-negative *Klebsiella* spp. *J Clin Microbiol* 2006;44(10):3640-3646. <https://doi.org/10.1128/JCM.00940-06>
11. van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption/ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. *J Clin Microbiol* 2010;48(3):900-907. <https://doi.org/10.1128/JCM.02071-09>
12. Martínez J, Martínez L, Rosenblueth M, Silva J, Martínez-Romero E. How are gene sequence analyses modifying bacterial taxonomy? The case of *Klebsiella*. *Int Microbiol* 2004;7(4):261-268.
13. Chun-Yan W, Li L, Li-Jing L, Yong-Xiu X, Chun-Jin H, Li-Tao Y, et al. Endophytic nitrogen-fixing *Klebsiella variicola* strain DX120E promotes sugarcane growth. *Biol Fertil Soils* 2014;50(4):657-666. <https://doi.org/10.1007/s00374-013-0878-3>
14. Fouts DE, Tyler HL, DeBoy RT, Daugherty S, Ren Q, Badger JH, et al. Complete genome sequence of the N₂-fixing broad host range endophyte *Klebsiella pneumoniae* 342 and virulence predictions verified in mice. *PLoS Genet* 2008;4(7):e1000141. <https://doi.org/10.1371/journal.pgen.1000141>

15. Medrano EG, Forray MM, Bell AA. Complete genome sequence of a *Klebsiella pneumoniae* strain isolated from a known cotton insect boll vector. *Genome Announc* 2014;2(4):pii: e00850-14. <https://doi.org/10.1128/genomeA.00850-14>
16. Rosenblueth M, Martinez L, Silva J, Martinez-Romero E. *Klebsiella variicola*, a novel species with clinical and plant-associated isolates. *Syst Appl Microbiol* 2004;27(1):27-35. <https://doi.org/10.1078/0723-2020-00261>
17. Maatallah M, Vading M, Kabir MH, Bakhrouf A, Kalin M, Naucler P, et al. *Klebsiella variicola* is a frequent cause of bloodstream infection in the stockholm area, and associated with higher mortality compared to *K. pneumoniae*. *PLoS One* 2014;9(11):e113539. <https://doi.org/10.1371/journal.pone.0113539>
18. Di DY, Jang J, Unno T, Hur HG. Emergence of *Klebsiella variicola* positive for NDM-9, a variant of New Delhi metallo- β -lactamase, in an urban river in South Korea. *J Antimicrob Chemother* 2017;72(4):1063-1067. <https://doi.org/10.1093/jac/dkw547>
19. Hopkins KL, Findlay J, Doumith M, Mather B, Meunier D, D'Arcy S, et al. IMI-2 carbapenemase in a clinical *Klebsiella variicola* isolated in the UK. *J Antimicrob Chemother* 2017;72(7):2129-2131. <https://doi.org/10.1093/jac/dkx103>
20. Breurec S, Melot B, Hoen B, Passet V, Schepers K, Bastian S, et al. Liver Abscess Caused by Infection with Community-Acquired *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*. *Emerg Infect Dis* 2016;22(3):529-531. <https://doi.org/10.3201/eid2203.151466>