

wzi Gene Sequencing, a Rapid Method for Determination of Capsular Type for *Klebsiella* Strains

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Pathogens of the genus *Klebsiella* have been classified into distinct capsular (K) types for nearly a century. K typing of *Klebsiella* species still has important applications in epidemiology and clinical microbiology, but the serological method has strong practical limitations. Our objective was to evaluate the sequencing of *wzi*, a gene conserved in all capsular types of *Klebsiella pneumoniae* that codes for an outer membrane protein involved in capsule attachment to the cell surface, as a simple and rapid method for the prediction of K type. The sequencing of a 447-nucleotide region of *wzi* distinguished the K-type reference strains with only nine exceptions. A reference *wzi* sequence database was created by the inclusion of multiple strains representing K types associated with high virulence and multidrug resistance. A collection of 119 prospective clinical isolates of *K. pneumoniae* were then analyzed in parallel by *wzi* sequencing and classical K typing. Whereas K typing achieved typeability for 81% and discrimination for 94.4% of the isolates, these figures were 98.1% and 98.3%, respectively, for *wzi* sequencing. The prediction of K type once the *wzi* allele was known was 94%. *wzi* sequencing is a rapid and simple method for the determination of the K types of most *K. pneumoniae* clinical isolates.

Bacteria of the genus *Klebsiella* are important pathogens. *Klebsiella pneumoniae*, the most prevalent species in human infections, is emerging as an agent of severe community infections, including pyogenic liver abscesses (1–3). Besides, multidrug-resistant infections by *K. pneumoniae* are increasingly reported and are responsible for countless outbreaks in health care settings (4). Strain typing is necessary to recognize clonal groups, to assist epidemiological investigations, and to link genetic diversity with pathophysiological specificities. Pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and capsular polysaccharide characterization (K typing) are widely used to characterize *K. pneumoniae* clinical isolates (4–7). MLST has been widely adopted as the common language for the characterization of *K. pneumoniae* and the recognition of important emerging sequence types (STs), such as ST23, which is associated with liver abscesses (8), and ST15, which is associated with multidrug resistance worldwide (9, 10). K typing was pioneered in 1926 (11), was further developed up until the 1970s (12), and still is a widely used characterization method. At least 78 K types can be distinguished for *K. pneumoniae*, some of which are strongly associated with virulence in experimental models (13) and with particular human infections (14, 15). However, K typing is technically difficult, and the production of the necessary reagents is complex. Molecular methods to deduce the K type from genomic sequences have been developed based on PCR restriction fragment length polymorphisms (PCR-RFLP) (16) or allele-specific PCR amplification (14, 17–19). Although the former method can be used to characterize most *K. pneumoniae* clinical isolates, it is technically complex. On the contrary, allele-specific PCRs are easy to perform but were mainly developed for serotypes that are frequent in community-acquired bacteremia and liver abscess isolates, namely, *K. pneumoniae* K1, K2, K5, K20, K54, and K57 (14, 17, 20, 21). However, these serotypes represent a small proportion (<20%) of *K. pneu-*

moniae isolates from sources other than liver abscesses or community-acquired pneumonia (6, 22, 23), thus leaving most of the classical *K. pneumoniae* isolates untypeable. Although it should be feasible to develop specific PCR assays for most K types, it is not practical given the limitations of PCR multiplexing. Furthermore, the occurrence of horizontal transfer between unrelated STs of the *cps* cluster that are responsible for capsular antigen synthesis and export implies that the K type cannot be predicted reliably from the ST despite a strong concordance between the two methods (24).

K-type variation has been linked to the presence or absence of particular genes at the *cps* locus (17, 25, 26). The *cps* locus has a mosaic structure, with a group of six conserved genes (*galF*, *orf2*, *wzi*, *wza*, *wzb*, and *wzc*) at its 5' end. Among these, the *wzi* gene codes for the outer membrane protein Wzi, which is involved in attachment of the capsular polysaccharide to the outer membrane (27, 28). The objective of this study was to evaluate the sequence polymorphisms of the *wzi* gene for strain typing and for inference of the serotypes of *K. pneumoniae* clinical isolates.

Received 20 July 2013 Returned for modification 16 August 2013

Accepted 24 September 2013

Published ahead of print 2 October 2013

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JCM.01924-13>.

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doi:10.1128/JCM.01924-13

MATERIALS AND METHODS

Strains. Reference strains of the 77 K types of the international serotyping scheme (12) were included. Strains of the *K. pneumoniae* K types K1 to K54 were obtained from the Collection de l'Institut Pasteur. Strains from K types higher than 54 were obtained from the WHO reference center. *K. pneumoniae* strain A1517 (17) was a gift from J.-T. Wang. Seventy-five strains representing important K types or STs were gathered from our previous studies (1, 5, 16, 24, 29). One hundred nineteen *K. pneumoniae* isolates from infected patients hospitalized in five university hospitals between 2008 and 2012 were included. Infections were community acquired (54%) or nosocomial (46%), as defined by their isolation before or after 48 h of hospitalization, respectively. The clinical isolate specimens were recovered from urine ($n = 42$ [36%]), blood ($n = 28$ [24%]), lower respiratory tract ($n = 19$ [16%]), abscesses ($n = 9$ [8%]), peritonitis, cholangitis, or other digestive system infections ($n = 6$), cerebrospinal fluid ($n = 1$), or other sources ($n = 4$).

DNA preparation. DNA templates were prepared by suspending a freshly grown colony in 200 μ l of purified water, heating at 94°C for 10 min, and submitting the extracts to microcentrifugation at $7,500 \times g$ for 5 min. The supernatants were stored at -20°C until use.

K typing. K typing was performed at the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* by counter-current immunoelectrophoresis (CCIE), using a modified version of the method described by Palfreyman (30). An extract was used as the antigen instead of a whole-cell suspension (31). The extract was heated only once for 1 h at 100°C before centrifugation. K typing was performed blindly with respect to the results of the *wzi* sequencing.

***rpoB* sequence analysis.** The sequence of a 501-bp internal portion of the *rpoB* gene was obtained as described previously (32).

PCR amplification and sequencing of *wzi* gene. PCR amplification of a 580-bp DNA fragment was achieved using the primers *wzi_for2* (GTG CCG CGA GCG CTT TCT ATC TTG GTA TTC C) and *wzi_rev* (GAG AGC CAC TGG TTC CAG AA[C or T] TT[C or G] ACC GC). Two microliters of the DNA extract was used as the template for PCR. The 50- μ l PCR mixture contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.2 μ M each primer, 100 μ M each deoxynucleoside triphosphate, and 0.17 μ l *Taq* polymerase (Invitrogen, France). After denaturation at 94°C for 2 min, 30 cycles were performed (each 94°C for 30 s, 55°C for 40 s, and 72°C for 30 s), followed by a final elongation step (72°C for 5 min). Sanger sequencing was performed on both strands using the PCR primers. An internal portion of 447 bp, which was well covered by sequence chromatograms on both strands, was retained for sequence comparisons. This selected region corresponded to the 5' region of the *wzi* gene, starting at the ATG initiation codon. The template used for *wzi* characterization thus corresponds to the 149 N-terminal amino acids of the outer membrane protein.

Data analysis. Forward and reverse chromatograms were assembled using BioNumerics version 6.6 (Applied Maths, Belgium). Phylogenetic trees were constructed using MEGA version 5.1 (33) based on the neighbor-joining method and Jukes-Cantor distance. The Simpson index of discrimination and the adjusted Wallace coefficient of concordance were computed using the Comparing Partitions tool (see <http://comparingpartitions.info>).

RESULTS AND DISCUSSION

Identification of the K-type reference strains. The international *Klebsiella* K-typing scheme was developed between 1916 and 1977 (11, 12). Subsequently, the taxonomy of *Klebsiella* has evolved with the proposal to reclassify three species into the genus *Raoultella* and with descriptions of the novel species *Klebsiella variicola* and *Klebsiella michiganensis* (34–37). To our knowledge, identification of the reference strains of the 77 K types at the species level has not been reevaluated in light of these taxonomic changes. To better characterize the phylogenetic range of the *wzi* primers used

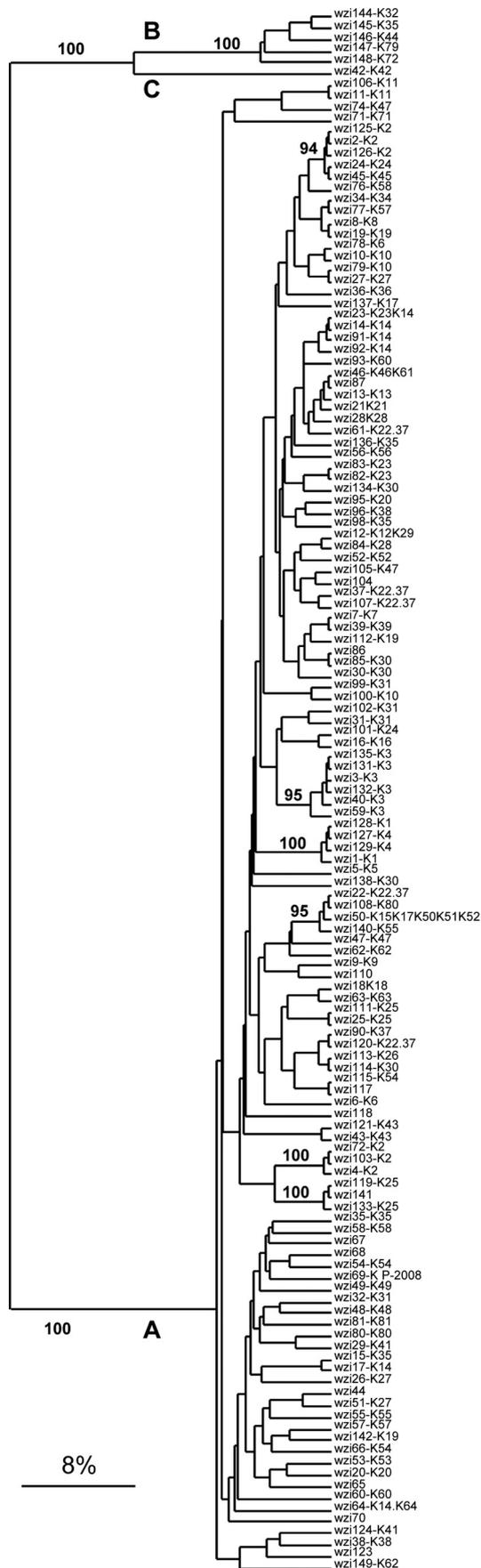
in this study (see below), we sequenced the *rpoB* gene of each K-type reference strain and compared them to the *rpoB* sequences previously described for taxonomic type strains (32, 34, 36–39). A portion of 501 nucleotides was sequenced from the 77 K-type reference strains. Based on the phylogenetic analysis of the *rpoB* gene sequence (see Fig. S1 in the supplemental material), the 77 reference strains were attributed to *K. pneumoniae* ($n = 54$), including three strains (K50, K60, and K80) corresponding to phylogenetic group KpII-B (40) and three strains (K4, K5, and K6) belonging to *K. pneumoniae* subsp. *ozaenae*. Other reference strains were attributed to *K. variicola* (K48, K49, K53, K54, K56, K57, K58, and K71), *Klebsiella oxytoca* (K26, K29, and K74), *K. michiganensis* (K41, K59, K66, and K70), *K. (Raoultella) planticola* (K35 and K79), *K. (Raoultella) ornithinolytica* (K32, K44, and K72), and *K. (Raoultella) terrigena* (K65, K67, K68, and K69).

***wzi* sequencing of K-type reference strains.** *wzi* is one of the few genes present in the capsular polysaccharide cluster of all capsular types of *K. pneumoniae*. The alignment of previously published capsular polysaccharide cluster sequences (17, 25, 26, 41, 42) allowed us to identify within the *wzi* gene a region of high sequence variability flanked by conserved motifs that are suitable for primer design. The 78 K-type reference strains, including strain A1517 (17), were analyzed by PCR using the primers *wzi_for2* and *wzi_rev*. For 64 (80%) reference strains, a PCR product of the expected size (580 bp) was obtained. The 14 reference strains that were PCR negative included the *K. oxytoca*, *K. michiganensis* (except K59), and *K. terrigena* strains. In contrast, PCR amplification was negative for only three (K33, K40, and K82) *K. pneumoniae* strains and none of the *K. variicola*, *K. planticola*, and *K. ornithinolytica* K-type reference strains. Negative PCR results might be due to priming site sequence variation or other alterations of the *wzi* gene. Note that PCR amplification of the *Escherichia coli* isolates that harbor group 1 capsule gene clusters can be positive, given the sequence similarity observed with *Klebsiella* clusters (43).

The *wzi* sequences obtained in this study are provided in File S3 in the supplemental material. Sequencing of the 64 *wzi* PCR products of the reference strains distinguished 58 *wzi* alleles (see Table S1 in the supplemental material). The reference strains of all K types had distinct *wzi* sequences, with the following exceptions: K46 and K61, K14 and K23, and the group of K15, K17, K50, K51, and K52. To our knowledge, these nine serotypes were not associated with severe infections or multidrug resistance.

A phylogenetic analysis showed three main branches (Fig. 1). The first branch (branch A) corresponded to all but one *K. pneumoniae* reference strain and also included *K. variicola* reference strains. Strain A1517 had a unique *wzi* sequence, which clustered close to the K54 reference strain. Branch B included the *wzi* sequences from *K. planticola* and *K. ornithinolytica* (strains K32, K35, K44, K72, and K79). Finally, branch C comprised the K42 reference strain only, which differed from the strains of group B by 14%, on average. The *wzi* sequences of groups B and C diverged from group A by 22%, on average.

It is significant that the *wzi* sequences of serotypes K1 and K4 are very closely related, differing by a single nucleotide position (*wzi*-128 and *wzi*-127) (Fig. 1; see also File S3 in the supplemental material). This result is consistent with the similarity of the capsular polysaccharide (*cps*) clusters of these two serotypes (16). Serotype K4 is a hallmark of *K. pneumoniae* subsp. *ozaenae*, which is associated with the chronic condition ozena. Other pairs of



closely related *wzi* sequences were (i) K24 and K45, (ii) K7 and K39, and (iii) K8 and K19 (Fig. 1; see also File S3 in the supplemental material).

***wzi* sequencing of multiple strains of medically important K types.** In order to determine the diversity of the *wzi* allele sequences among strains of the same K type, we sequenced *wzi* for a total of 75 strains whose K types were previously determined. These included 43 multiple isolates of serotypes known to be associated with rhinoscleroma and ozaenae (K3 and K4), with liver abscesses and other community-acquired invasive infections (K1, K2, K5, K20, K54, and K57), and with multidrug resistance (ST15). Table S1 in the supplemental material provides details on the correspondence between K types, STs, and *wzi* alleles.

Four *K. pneumoniae* K1 isolates, including strain NTUH-K2044, were included. These four isolates belong to ST23, the liver abscess-associated clone (8, 24), whereas the reference strain of serotype K1 (CIP 52.208 = A5054) belongs to the nonvirulent clone ST82 (24). The four ST23 K1 isolates had identical *wzi* sequences (*wzi-1*), which differed by two nucleotides from that of CIP 52.208 (*wzi-128*) (see File S3 in the supplemental material). These results suggest that *wzi* sequencing can distinguish ST23 strains.

Seven *K. pneumoniae* K2 isolates were sequenced at the *wzi* gene. These isolates were selected to represent ST86 and ST380, which are associated with severe infections (1), and the nonvirulent ST14 clone (24). These K2 isolates also differed by their *cps* PCR-RFLP patterns, which were C patterns C2b to C2e (16). Nevertheless, the seven *wzi* sequences turned out to be totally identical (allele *wzi-2*). Surprisingly, this *wzi* sequence was very distinct (27 nucleotide [nt] differences out of 447 [6.0%]) from the *wzi* sequence (allele *wzi-4*) of the *K. pneumoniae* K2 reference strain B5055 (= CIP 52.145). The seven *wzi-2* isolates differed by a single nucleotide from eight isolates of serotype K24 and the reference strain of this serotype, which were identical (allele *wzi-24*). The K24 isolates belong to ST15, a widely distributed multiresistant clone of *K. pneumoniae* (9, 10, 24). Therefore, *wzi* sequencing might help in the identification of the international clone ST15 of serotype K24.

Six K3 isolates were included. These included two *K. pneumoniae* subsp. *rhinoscleromatis* strains of ST67 and four *K. pneumoniae* subsp. *pneumoniae* strains of ST8, ST13, ST71, and ST153. These isolates represented all known K3 variant C patterns, C3b to C3d (16). Remarkably, all six isolates clustered in a single branch together with the K3 reference strain CIP 52.146 (ST3). This branch was specific to K3 strains and differed by >4.5% from any other *wzi* sequence.

Three K4 strains of *K. pneumoniae* subsp. *ozaenae* were included. Their *wzi* sequence (*wzi-129*) clustered in a tight branch (99.7%) with the K4 reference strain (CIP 52.211 = D5050) and with the K1 reference strain. This cluster was closely related (99.34%) to *wzi-1*, the K1 ST23 *wzi* sequence.

FIG 1 Dendrogram of 135 *wzi* alleles. The tree was built using BioNumerics version 6.6 based on the uncorrected *p* distance. Each sequence corresponds to a distinct *wzi* allele, as indicated. The allele number is followed by the corresponding capsular type(s). Dots separating several K types indicate cross-reactions, whereas successions of distinct K types with no dot (e.g., K15K17K50K51K52) indicate that the *wzi* allele was found in strains reacting individually with one of these distinct K types. The three main branches (A, B, and C) are labeled. K P-2008 indicates the novel serotype described by Pan et al. (17). Bootstrap values of the remarkable nodes are given.

Four K5 isolates were included and had an identical *wzi* sequence (allele *wzi-5*) to that of the K5 reference strain. The *wzi-5* sequence differed from any other *wzi* sequence by >5%, underlining its distinctness.

We included three K20 isolates, one of which showed a *wzi* sequence identical to that of the K20 reference strain, whereas the two others had very distinct *wzi* alleles (31 nt differences, 6.9%). Both *wzi* alleles were unique to the K20 isolates.

Of six K54 isolates, two isolates had *wzi-115*, which differed by 7% from the *wzi* sequence (*wzi-54*) of the K54 reference. The *wzi* sequence (*wzi-66*) of the four other isolates was distinct, with 4% divergence to any other *wzi* allele.

Two isolates of K57 had identical *wzi* sequences (*wzi-77*), which were distinct from the *wzi* sequence of the K57 reference strain identified as *K. variicola*. Allele *wzi-77* was closely related (1 nucleotide difference [99.78%]) to the *wzi* sequence of the reference strain of K34 (Fig. 1; also see File S3 in the supplemental material).

***wzi* sequencing of additional strains of known K types.** In order to supplement the database of the reference *wzi* sequences, we included 32 strains that represented a variety of C patterns (16) and whose K types were known. These strains showed 25 distinct *wzi* alleles, 17 (68%) of which were novel compared to the 109 strains described above, thus supplementing the database of reference *wzi* sequences. The *wzi* alleles from these strains and those of the medically important K types described in the previous paragraph are given in Table S1 in the supplemental material.

***wzi* sequencing and K typing of prospective clinical isolates.** We aimed to compare the abilities of *wzi* sequencing and K typing to characterize clinical isolates. To this purpose, 119 isolates from community-acquired and nosocomial infections prospectively collected in five university hospitals were included. Of the 119 clinical isolates, 23 (19%) could not be typed by the serological method, resulting in a typeability rate of 81% for K typing. There were 35 distinct K types among the 96 typeable isolates, the most frequent ones being K2 ($n = 15$), K14 ($n = 11$), K1 ($n = 9$), and K51 ($n = 7$). Simpson's discrimination index was 94.4% (95% confidence interval, 92.3 to 96.6%). The *wzi* alleles obtained are described in Table S1 in the supplemental material.

Of the 119 isolates, only two isolates (T2 [nontypeable] and T17 [K2 isolate]) could not be PCR amplified for *wzi*; the 117 remaining isolates were successfully sequenced. Therefore, the typeability rate of *wzi* sequencing was 98.3%. A total of 72 distinct *wzi* alleles were found among the 117 clinical isolates, with a Simpson's discrimination index of 98.1% (confidence interval [CI], 97.1 to 99.0%). For the 95 isolates for which both the *wzi* sequence and the K type could be determined, there were 35 distinct K types (Simpson index, 94.6%; CI, 92.6 to 96.7%) and 55 *wzi* alleles (Simpson index, 97.4%; CI, 96.1 to 98.7%). Therefore, *wzi* sequencing was significantly more discriminatory and was able to type a much higher proportion of clinical isolates.

Of the 117 isolates, 61 (52%) had a *wzi* sequence that was identical to an allele in the reference database, corresponding to 25 distinct *wzi* alleles. Seven (11%) of them were nontypeable by K typing, and the remaining isolates were split into 20 distinct K types, among which K1 ($n = 9$), K14 ($n = 9$), K2 ($n = 8$), and K51 ($n = 7$) were the most common. There were six (10%) isolates for which *wzi* sequencing and K typing had discrepant results. First, one isolate identified as K29 had a *wzi* sequence identical to that of the K12 reference strain; this discrepancy was confirmed upon

retesting. Second, five isolates had allele *wzi-64*, which was the allele of K64 reference strain, whereas initial K typing (performed blindly) suggested they were K14 (four strains) or K22 (one isolate). However, as all five *wzi-64* isolates consistently reacted with K14 antiserum or with K64 antiserum (two isolates) upon retesting, we consider *wzi-64* to be indicative of K14 or K64 (thus denoted K14.K64).

The remaining 56 isolates for which the *wzi* sequence was not matched to the reference database represented 48 novel *wzi* alleles. Phylogenetic analysis showed that they all fell in branch A. Only six alleles were represented by more than one isolate (3 isolates had 2 alleles and 2 isolates had 4 alleles), showing that they were all uncommon. K typing showed that these isolates included 15 (26%) nontypeable isolates and 22 distinct K types. Importantly, among those, there were six K2 isolates. Their *wzi* sequences were very similar to the K2 *wzi* reference sequences *wzi-4* (four isolates, 2- or 3-nucleotide differences) or *wzi-2* (2 isolates, single-nucleotide difference), showing that K2 isolates can be recognized despite their sequence heterogeneity.

Considering the 48 novel *wzi* alleles to be novel reference sequences of their respective K type for future studies, the overall concordance among the two methods was estimated using the Wallace coefficient adjusted for agreement due to chance alone (44). The resulting directional agreement of K-type classification once knowing the *wzi* allele was 94.4% (CI, 91.1 to 97.7%). This result indicates that the K type can be predicted from a *wzi* allele in a large majority of cases. Conversely, knowing the K type predicted the *wzi* allele only weakly (adjusted Wallace coefficient, 44.1%; CI, 33.5 to 54.7%), as was expected by the higher diversity of *wzi* alleles than the K types.

Conclusions. This study reports the development of a rapid method, *wzi* sequencing, to predict the K types of *K. pneumoniae* isolates. PCR amplification was negative for K-type reference strains of several *Klebsiella* species but was positive for most strains of *K. pneumoniae* and *K. variicola*, which are the most frequently encountered *Klebsiella* species in clinical isolates (29, 40, 45, 46). The *wzi* sequences of most K-type reference strains were distinguished, with few exceptions. Besides, *wzi* sequences from multiple strains of important K types fell into recognizable clusters. The typeability rate of *wzi* sequencing was 98.3%, which is much higher than that of allele-specific PCR assays, which specifically target serotypes associated with liver abscesses or other community-acquired infections (14, 17, 20, 21). Based on the present database, the ability to predict the K type from the *wzi* sequence was estimated at nearly 95%. Furthermore, it should be noted that K-type reference strains are old and are likely to represent the current population of *K. pneumoniae* clinical isolates poorly. Therefore, it can be expected that typeability will improve by including additional clinical isolates in the reference database. Given that isolates with distinct K types can have closely related *wzi* alleles, we recommend that isolates with novel alleles be K typed and incorporated into the reference database. We are currently developing a BIGSdb genome database (47) into which the *wzi*-K-type correspondence will be incorporated and continuously updated. This will allow for the automated extraction of *wzi* allele information and the deduced K type from genomic sequences obtained using high-throughput sequencing technologies. Unfortunately, the K type cannot always be deduced based on *wzi* given sequence identities of some strains with distinct K types. It is expected that sequence information from other loci of the capsular polysaccha-

ride synthesis cluster could be used to resolve these cases. Nevertheless, *wzi* sequencing represents a clear improvement over the molecular methods that are currently available for approximating the capsular serotypes of *K. pneumoniae* isolates. Irrespective of K-typing correspondence, *wzi* sequencing also represents a powerful strain typing method *per se*, given its high discrimination and typeability achievements. It is our hope that this method will contribute to a better understanding of the epidemiology of groups of strains of particular medical importance, including hypervirulent and multidrug-resistant clonal groups, and will at the same time allow for linking future work to the large historical corpus of knowledge accumulated based on classical K typing.

ACKNOWLEDGMENTS

We thank Jin-Town Wang for providing strain A1517 and Karen A. Krogfelt and Flemming Scheutz for their helpful advice.

Financial support for this work was provided by a grant from Assistance Publique-Hôpitaux de Paris, contract CRC 08007 (KPath) and from the French government's Investissement d'Avenir program, Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant ANR-10-LABX-62-IBEID).

REFERENCES

- Decré D, Verdet C, Emirian A, Le Gourrierec T, Petit JC, Offenstadt G, Maury E, Brisse S, Arlet G. 2011. Emerging severe and fatal infections due to *Klebsiella pneumoniae* in two university hospitals in France. *J. Clin. Microbiol.* 49:3012–3014.
- Shon AS, Bajwa RP, Russo TA. 2013. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. *Virulence* 4:107–118.
- Wang JH, Liu YC, Lee SS, Yen MY, Chen YS, Wang JH, Wann SR, Lin HH. 1998. Primary liver abscess due to *Klebsiella pneumoniae* in Taiwan. *Clin. Infect. Dis.* 26:1434–1438.
- Woodford N, Turton JF, Livermore DM. 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol. Rev.* 35:736–755.
- Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J. Clin. Microbiol.* 43:4178–4182.
- Hansen DS, Skov R, Benedi JV, Sperling V, Kolmos HJ. 2002. *Klebsiella* typing: pulsed-field gel electrophoresis (PFGE) in comparison with O:K-serotyping. *Clin. Microbiol. Infect.* 8:397–404.
- Vimont S, Mnif B, Fevre C, Brisse S. 2008. Comparison of PFGE and multilocus sequence typing for analysis of *Klebsiella pneumoniae* isolates. *J. Med. Microbiol.* 57:1308–1310.
- Turton JF, Engender H, Gabriel SN, Turton SE, Kaufmann ME, Pitt TL. 2007. Genetically similar isolates of *Klebsiella pneumoniae* serotype K1 causing liver abscesses in three continents. *J. Med. Microbiol.* 56:593–597.
- Damjanova I, Tóth A, Pászti J, Hajbel-Vékony G, Jakab M, Berta J, Milch H, Füzi M. 2008. Expansion and countrywide dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-type beta-lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005—the new 'MRSA's'? *J. Antimicrob. Chemother.* 62:978–985.
- Hu L, Zhong Q, Tu J, Xu Y, Qin Z, Parsons C, Zhang B, Hu X, Wang L, Yu F, Pan J. 2013. Emergence of *bla*_{NDM-1} among *Klebsiella pneumoniae* ST15 and novel ST1031 clinical isolates in China. *Diagn. Microbiol. Infect. Dis.* 75:373–376.
- Julianelle LA. 1926. Immunological relationships of encapsulated and capsule-free strains of *Encapsulatus pneumoniae* (Friedländer's bacillus). *J. Exp. Med.* 44:683–696.
- Ørskov I, Ørskov F. 1984. Serotyping of *Klebsiella*. *Methods Microbiol.* 14:117–133.
- Mizuta LG, Ohta M, Mori M, Hasegawa T, Nakashima I, Kato N. 1983. Virulence for mice of *Klebsiella* strains belonging to the O1 group: relationship to their capsular (K) types. *Infect. Immun.* 40:56–61.
- Fang CT, Lai SY, Yi WC, Hsueh PR, Liu KL, Chang SC. 2007. *Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. *Clin. Infect. Dis.* 45:284–293.
- Fung CP, Chang FY, Lee SC, Hu BS, Kuo BI, Liu CY, Ho M, Siu LK. 2002. A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis? *Gut* 50:420–424.
- Brisse S, Issenhuth-Jeanjean S, Grimont PA. 2004. Molecular serotyping of *Klebsiella* species isolates by restriction of the amplified capsular antigen gene cluster. *J. Clin. Microbiol.* 42:3388–3398.
- Pan YJ, Fang HC, Yang HC, Lin TL, Hsieh PF, Tsai FC, Keynan Y, Wang JT. 2008. Capsular polysaccharide synthesis regions in *Klebsiella pneumoniae* serotype K57 and a new capsular serotype. *J. Clin. Microbiol.* 46:2231–2240.
- Struve C, Bojer M, Nielsen EM, Hansen DS, Krogfelt KA. 2005. Investigation of the putative virulence gene *magA* in a worldwide collection of 495 *Klebsiella* isolates: *magA* is restricted to the gene cluster of *Klebsiella pneumoniae* capsule serotype K1. *J. Med. Microbiol.* 54:1111–1113.
- Yu WL, Fung CP, Ko WC, Cheng KC, Lee CC, Chuang YC. 2007. Polymerase chain reaction analysis for detecting capsule serotypes K1 and K2 of *Klebsiella pneumoniae* causing abscesses of the liver and other sites. *J. Infect. Dis.* 195:1235–1236.
- Yeh KM, Kurup A, Siu LK, Koh YL, Fung CP, Lin JC, Chen TL, Chang FY, Koh TH. 2007. Capsular serotype K1 or K2, rather than *magA* and *rmpA*, is a major virulence determinant for *Klebsiella pneumoniae* liver abscess in Singapore and Taiwan. *J. Clin. Microbiol.* 45:466–471.
- Yu VL, Hansen DS, Ko WC, Sagnimeni A, Klugman KP, Goossens H, Wagener MM, Benedi VJ. 2007. Virulence characteristics of *Klebsiella* and clinical manifestations of *K. pneumoniae* bloodstream infections. *Emerg. Infect. Dis.* 13:986–993.
- Cryz SJ, Jr, Mortimer PM, Mansfield V, Germanier R. 1986. Seroepidemiology of *Klebsiella pneumoniae* bacteremic isolates and implications for vaccine development. *J. Clin. Microbiol.* 23:687–690.
- Toivanen P, Hansen DS, Mestre F, Lehtonen L, Vaahotvu J, Vehma M, Möttönen T, Saario R, Luukkainen R, Nissilä M. 1999. Somatic serogroups, capsular types, and species of fecal *Klebsiella* in patients with ankylosing spondylitis. *J. Clin. Microbiol.* 37:2808–2812.
- Brisse S, Fevre C, Passet V, Issenhuth-Jeanjean S, Tournebize R, Diancourt L, Grimont P. 2009. Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. *PLoS One* 4:e4982. doi:10.1371/journal.pone.0004982.
- Arakawa Y, Wacharotayankun R, Nagatsuka T, Ito H, Kato N, Ohta M. 1995. Genomic organization of the *Klebsiella pneumoniae* cps region responsible for serotype K2 capsular polysaccharide synthesis in the virulent strain Chedid. *J. Bacteriol.* 177:1788–1796.
- Fevre C, Passet V, Deletoile A, Barbe V, Frangeul L, Almeida AS, Sansonetti P, Tournebize R, Brisse S. 2011. PCR-based identification of *Klebsiella pneumoniae* subsp. *rhinoscleromatis*, the agent of rhinoscleroma. *PLoS Negl. Trop. Dis.* 5:e1052. doi:10.1371/journal.pntd.0001052.
- Bushell SR, Mainprize IL, Wear MA, Lou H, Whitfield C, Naismith JH. 2013. Wzi is an outer membrane lectin that underpins group 1 capsule assembly in *Escherichia coli*. *Structure* 21:844–853.
- Rahn A, Beis K, Naismith JH, Whitfield C. 2003. A novel outer membrane protein, Wzi, is involved in surface assembly of the *Escherichia coli* K30 group 1 capsule. *J. Bacteriol.* 185:5882–5890.
- Brisse S, Verhoef J. 2001. Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, *gyrA* and *parC* genes sequencing and automated ribotyping. *Int. J. Syst. Evol. Microbiol.* 51:915–924.
- Palfreyman JM. 1978. *Klebsiella* serotyping by counter-current immunoelectrophoresis. *J. Hygiene (Lond.)* 81:219–225.
- Orskov F, Orskov I. 1972. Immunoelectrophoretic patterns of extracts from *Escherichia coli* O antigen test strains O1 to O157 examinations in homologous OK sera. Further comments on the classification of *Escherichia* K antigens. *Acta Pathol. Microbiol. Scand. B Microbiol. Immunol.* 80:905–910.
- Salerno A, Delétoile A, Lefevre M, Ciznar I, Krovacek K, Grimont P, Brisse S. 2007. Recombining population structure of *Plesiomonas shigelloides* (*Enterobacteriaceae*) revealed by multilocus sequence typing. *J. Bacteriol.* 189:7808–7818.
- Tamura K, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28:2731–2739.
- Drancourt M, Bollet C, Carta A, Roussetier P. 2001. Phylogenetic analyses of *Klebsiella* species delineate *Klebsiella* and *Raoultella* gen. nov.,

- with description of *Raoultella ornithinolytica* comb. nov., *Raoultella terrigena* comb. nov. and *Raoultella planticola* comb. nov. *Int. J. Syst. Evol. Microbiol.* 51:925–932.
35. Grimont PAD, Grimont F. 2005. Genus *Klebsiella*, p 685–693. In Brenner DJ, Krieg NR, Staley JT (ed), *Bergey's manual of systematic bacteriology*, vol 2: the proteobacteria, part B: the gammaproteobacteria. Springer-Verlag, New York, NY.
 36. Rosenblueth M, Martínez L, Silva J, Martínez-Romero E. 2004. *Klebsiella variicola*, a novel species with clinical and plant-associated isolates. *Syst. Appl. Microbiol.* 27:27–35.
 37. Saha R, Farrance CE, Verghese B, Hong S, Donofrio RS. 2013. *Klebsiella michiganensis* sp. nov., a new bacterium isolated from a tooth brush holder. *Curr. Microbiol.* 66:72–78.
 38. Delétoile A, Decré D, Courant S, Passet V, Audo J, Grimont P, Arlet G, Brisse S. 2009. Phylogeny and identification of *Pantoea* species and typing of *Pantoea agglomerans* strains by multilocus gene sequencing. *J. Clin. Microbiol.* 47:300–310.
 39. Paauw A, Caspers MP, Schuren FH, Leverstein-van Hall MA, Delétoile A, Montijn RC, Verhoef J, Fluit AC. 2008. Genomic diversity within the *Enterobacter cloacae* complex. *PLoS One* 3:e3018. doi:10.1371/journal.pone.0003018.
 40. Fevre C, Passet V, Weill FX, Grimont PA, Brisse S. 2005. Variants of the *Klebsiella pneumoniae* OKP chromosomal beta-lactamase are divided into two main groups, OKP-A and OKP-B. *Antimicrob. Agents Chemother.* 49:5149–5152.
 41. Wu KM, Li LH, Yan JJ, Tsao N, Liao TL, Tsai HC, Fung CP, Chen HJ, Liu YM, Wang JT, Fang CT, Chang SC, Shu HY, Liu TT, Chen YT, Shiau YR, Lauderdale TL, Su IJ, Kirby R, Tsai SF. 2009. Genome sequencing and comparative analysis of *Klebsiella pneumoniae* NTUH-K2044, a strain causing liver abscess and meningitis. *J. Bacteriol.* 191:4492–4501.
 42. Shu HY, Fung CP, Liu YM, Wu KM, Chen YT, Li LH, Liu TT, Kirby R, Tsai SF. 2009. Genetic diversity of capsular polysaccharide biosynthesis in *Klebsiella pneumoniae* clinical isolates. *Microbiology* 155:4170–4183.
 43. Rahn A, Drummelsmith J, Whitfield C. 1999. Conserved organization in the *cps* gene clusters for expression of *Escherichia coli* group 1 K antigens: relationship to the colanic acid biosynthesis locus and the *cps* genes from *Klebsiella pneumoniae*. *J. Bacteriol.* 181:2307–2313.
 44. Severiano A, Pinto FR, Ramirez M, Carrico JA. 2011. Adjusted Wallace coefficient as a measure of congruence between typing methods. *J. Clin. Microbiol.* 49:3997–4000.
 45. Brisse S, van Himbergen T, Kusters K, Verhoef J. 2004. Development of a rapid identification method for *Klebsiella pneumoniae* phylogenetic groups and analysis of 420 clinical isolates. *Clin. Microbiol. Infect.* 10:942–945.
 46. Podschun R, Ullmann U. 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev.* 11:589–603.
 47. Jolley KA, Maiden MC. 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* 11:595. doi:10.1186/1471-2105-11-595.