Characterization of hypervirulent Klebsiella pneumoniae isolates in Belgium

Anantharajah, Ahalieyah; Deltombe, Matthieu; de Barsy, Marie; Evrard, Stephanie; Denis, Olivier; Bogaerts, Pierre; Hallin, Marie; Miendje Deyi, Véronique Yvette; Pierard, Denis; Bruynseels, Peggy; Boelens, Jerina; Glupczynski, Youri; Huang, Te-Din

Published in:
European Journal of Clinical Microbiology & Infectious Diseases : Official Publication of the European Society of Clinical Microbiology

DOI:
10.1007/s10096-022-04438-z

Publication date:
2022

Document Version:
Accepted author manuscript

Brief report (EJCMID)

Characterization of Hypervirulent *K. pneumoniae* Isolates in Belgium.

Ahalieyah Anantharajah[1,2,*], Matthieu Deltombe[1], Marie De Barsy[1], Stephanie Evrard[1], Olivier Denis[1], Pierre Bogaerts[1], Marie Hallin[3], Véronique Yvette Miendje Deyi[3], Denis Pierard[4], Bart Gordts[5], Jerina Boelens[6], Youri Glupczynski[1], Te-Din Huang[1]

[1]Department of Clinical Microbiology, National reference center for antibiotic-resistant Gram-negative bacilli, CHU UCL Namur, Yvoir, Belgium

[2]Current affiliation: Department of Clinical Microbiology, Cliniques Universitaires Saint-Luc, UCL, Brussels, Belgium

[3]Department of Microbiology, Laboratoire Hospitalier Universitaire de Bruxelles (LHUB-ULB), Brussels, Belgium

[4]Department of Microbiology, Universiteit Ziekenhuis Brussel, Brussels, Belgium

[5]Department of Microbiology, Ziekenhuis Netwerk Antwerpen, Antwerp, Belgium.

[6]Department of Microbiology, Ghent University Hospital, Ghent, Belgium.

*__Corresponding author__: ahalieyah.anantharajah@uclouvain.be

Words : 1451 (max 1500)

Abstract (max 100 words) **(92 words)**

Hypervirulent *K. pneumoniae* (hvKp) raised concern worldwide. We studied 22 hvKp clinical invasive isolates referred to the Belgian national reference laboratory between 2014 and 2020. 64% of the isolates expressed K2 capsular serotype and belonged to 7 different MLST lineages, while 22% expressed K1 (all belonging to ST23) and were associated with liver abscesses. Primary extra-hepatic infections were reported in 36% and sepsis for 95% of the patients with 30% of deaths. Improved clinical and microbiological diagnostics are required as hvKp may represent an underestimated cause of community-acquired invasive infections in Belgium.

Keywords: Klebsiella pneumoniae, hypervirulence, hypermucoviscosity, whole genome sequencing, molecular epidemiology

Introduction:

A hypervirulent variant of *K. pneumoniae* was reported in the mid-1980s and has raised concerns in recent years due to its extraordinary invasiveness, resulting in higher morbidity and mortality [1]. Typically, hypervirulent *K. pneumoniae* (hvKp) are responsible for severe monomicrobial community-acquired infections such as cryptogenic pyogenic liver abscesses [2] often associated with unusual septic metastatic localizations, including endophthalmitis [3], or meningitis [4,5], in immunocompetent hosts of any age.

According to the current knowledge, no single factor can fully define hvKp [6]. The diagnosis of hvKp infection is based on a combination of clinical suspicion and microbiological characteristics of hvKp distinguishable from classic *K. pneumoniae* (cKp). Hypermucoviscosity representing the major feature for hypervirulence results from the overexpression of capsular polysaccharides. It confers to hvKp higher resistance to phagocytosis and intracellular killing by neutrophils and bactericidal complements. The most common capsule locus associated with hvKp is K1, followed by K2, K5 and K57 [1,7]. Most of hvKp strains of K1 serotype belong to clonal complex (CC)23, while K2 serotype strains belong to multiple distinct multilocus sequence types (MLSTs) [8]. hvKp has acquired virulence genes present on large plasmids (e.g., pK2044 and pLVPK) and within integrative conjugative elements that participate to its hypervirulent phenotype such as the mucoid phenotype regulator involved in capsular synthesis and the iron acquisition systems and siderophores (e.g., aerobactin salmochelin, yersiniabactin) involved in growth and survival of bacteria [1,9].

While most cases have been initially described from South-East Asia, increasing reports have since evidenced hvKp infections worldwide. The estimated prevalence of hvKp among *K. pneumoniae* bacteraemia isolates was 8.2% in Canada [10], 6.3% in the USA [11] and 2.8% in Spain [12]. Yet, there have been no studies reported on the burden and characterization of hvKp in Belgium. We investigated retrospectively clinical and microbiological characteristics of hvKp strains collected by the Belgian National Reference Center for Antibiotic-Resistant Gram-Negative Bacilli (NRC) between 2014 and 2020.

Materials and Methods:

Between January 2014 and January 2020, a total of 22 putative hypervirulent *K. pneumoniae* isolates were referred to NRC based on clinical presentation. The bacterial isolates were collected from distinct patients dispersed in eight Belgian hospitals. Antimicrobial susceptibility testing was performed by disc diffusion using EUCAST interpretative breakpoints [13].

All isolates were tested for the hypermucoviscous phenotype by performing the ‘string test’ (the formation of a viscous string >5 mm in length when fresh bacterial colonies are stretched

by an inoculation loop [14]) and analysed by whole genome sequencing (WGS). Libraries were constructed with Nextera DNA Flex Library Prep Kit (Illumina Inc, CA, USA) and were sequenced on the Illumina iSeq according to the manufacturer's protocol. *Klebsiella pneumoniae* taxonomy ID (573) was confirmed by Kraken 2 [15]. Resistance genes were identified with ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/). Virulence genes, MLST and strict core genome MLST (scg-MLST) were determined using Institut Pasteur MLST databases (https://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html) [16] while capsule type using Kaptive (http://kaptive.holtlab.net/). Whole-genome MLST (wgMLST) was analysed using BioNumerics (version 8.0, Applied-Maths, St Martens Latem, Belgium) with a scheme containing 19.729 loci for *K. pneumoniae*. To detect the virulence plasmids, the contigs of hvKp isolates were aligned with well-characterized virulence plasmids pLVPK (GenBank accession AY378100) [17], pK0244 (GenBank accession AP006726.1) [18], Kp52.145 plasmid I and Kp52.145 plasmid II (GenBank accession FO834905.1) [19].

Results and Discussion:

Of the 22 hvKp clinical isolates, 7 and 14 isolates were identified as capsular genotypes K1 and K2, respectively. While studies in other countries have reported various prevalence rate of K57 serotype [20,21], only one case was observed in this study.

Clinical records available are summarized in **Table 1**. All patients developed monomicrobial, community-acquired invasive infections. The median age was 61.3 years, with a majority of male patients (17/22) [20]. Underlying disorders such as diabetes mellitus, hepatobiliary diseases, active neoplasia, human immunodeficiency virus infection, alcoholism and benign prostatic hyperplasia were reported in the majority (85%; 17/20; no information for two patients) of infected patient and are consistent with previous reports [2,22-24]. Birthplace and travel information when available (19/22) indicated that one Asian patient travelled to Hong Kong before infection, one came from Romania, seven were from African countries and ten were born in Belgium without notion of travelling abroad. Unlike previous reports [2,23], Asian patients did not represent the main ethnic group (only one case (5%; 1/22) in our study vs. 50% (16/32) in cohorts in the United States [2]) suggesting the successful establishment of this pathogen in Belgium causing invasive infections among ethnicities other than Asian communities.

Anatomical origins of hvKp isolates included peripheral blood (72.8%; 16/22), hepato-biliary (13.7%; 3/22), ocular (4.5%; 1/22), genital (4.5%; 1/22) and soft tissues (4.5%; 1/22). The primary site of infection was a liver abscess for 63.6% (14/22) of the patients. Of note, the K1-

isolates were exclusively associated with liver abscesses. Extra-hepatic manifestations in lung (pneumonia or lung abscess) were reported in five patients, while endophthalmitis, necrotising fasciitis and orchiepididymitis were incriminated once each as the primary infection site. Sepsis occurred during the course of the infection for more than 95% of the patients. Septic metastatic foci were documented only for three patients, but could be underreported if extensive anatomical investigations were not conducted. Life-threatening conditions required admission to an intensive care unit during their hospital stay for nearly half (10/22) of the patients and 30% (6/20; no information for two patients) died. Of these deaths, all occurred in infections by non-K1 isolates and five were associated with primary extrahepatic manifestations. Despite the lack of recommendations of the most suitable antibiotics for treating hvKp infections, patients were mostly treated with antibiotics such as penicillin/beta-lactamase inhibitor, cephalosporin and fluoroquinolone alone or in combination, associated in ten cases with abscess drainage in accordance with previous studies [2].

MLST revealed that K1 isolates (n=7) belonged exclusively to ST23 and K57 isolate belonged to ST592 whereas K2 isolates arose from seven distinct clonal lineages (ST380 (n=3), ST2039 (n=3), ST65 (n=3), ST66 (n=2), ST25 (n=1), ST86 (n=1), ST3240 (n=1)), confirming the phylogenetic diversity within the K2 isolates **(Fig 1**). In addition to those previously described [21,25], we reported here a K2 isolates from ST3240. New hvKp STs continue to emerge indicated the ongoing evolution of hvKp [8,25,26]. The 22 isolates were associated with hypermucoviscous phenotype and positive string test. Of note, hypermucoviscous cKp (non-hypervirulent) strains and hvKp strains without hypermucoviscosity have been described [27].

All K1/ST23 isolates were positive for the virulence genes: *rmpA* and *rmpA2* mucoid phenotype regulator, yersiniabactin (*ybt),* aerobactin (i*utA* and *iuc)* and salmochellin (*iro*) siderophores, *kfu* iron uptake, *clb* colibactin toxin, *mrk* type III fimbriae, and *allS* for allantoin metabolism. The non-K1 isolates possessed also *rmpA gene, iuc, iut, iro and mrk* loci but lacked *allS* gene. *rmpA2, ybt, clb* and *kfu* loci were variably present in non-K1 isolates. Isolates belonging to a specific ST showed identical virulence gene profiles. Blast analysis identified the presence of highly similar (≥99% identity and ≥90% coverage) virulence pLVPK–like plasmid in ST23 and pK2044-like plasmid in ST65, ST86, ST3240 and ST592 isolates. While two plasmids, similar to Kp52.145 plasmid I and Kp52.145 plasmid II [28] were identified in ST66 and ST380 isolates, only the Kp52.145 plasmid II was detected in ST2039. None of these plasmids was identified in ST25 isolate. All the strains showed a wild-type *in vitro* susceptibility profile toward beta-lactams without any acquired resistance other than ampicillin resistance mediated by the presence of bla_{SHV}. Interestingly, the two ST66 isolates did not

carry the intrinsic *bla*$_{SHV}$ explaining the exceptional *in vitro* susceptibility to ampicillin observed in these isolates [28].

Increasing reports have described the emergence of multidrug-resistant hvKp strains. Carbapenem-resistant hvKp, initially described in China, are reported all over the world with risk assessment required to evaluate and increase the awareness of the potential significant threat to public health [29,30].

As hvKp isolates and clinical information for the corresponding patients were referred to the NRC on a voluntary basis by the hospital laboratories, our current study may not perfectly reflect the Belgian epidemiology. Nevertheless, our study evidenced the predominant circulation in Belgium of genetically diverse hvKp expressing capsular type K2 among local population unrelated to Asian ethnicity. These data suggest a potential underestimated spread of such invasive isolates. Further epidemiological studies of hvKp with a structured national survey are needed.

Increased clinical awareness among physicians facing invasive or recurrent infections caused by *K. pneumoniae* should incite prompt microbiological diagnostic based on hypermucoviscous phenotype and differentiation of hvKp from cKp by molecular characterization of major virulence factors. Virulence genes commonly found in hvKp strains (e.g. *rmpA*) might serve as genotypic biomarkers for the early detection of hvKp strains in areas of low prevalence [31]. Management challenges included rapid initiation of therapy to prevent subsequent spread and sepsis, investigation for occult abscess to enable source control and appropriate site-targeted therapy.

Funding

The Belgian national reference center for antibiotic-resistant Gram-negative bacilli is supported in part by the Belgian Ministry of Social Affairs through a fund within the National health insurance system (INAMI-RIZIV).

Conflicts of interest

The authors declare that they have no conflict of interest.

Informed Consent Statement

Not applicable

References:

1. Russo TA, Marr CM (2019) Hypervirulent Klebsiella pneumoniae. Clin Microbiol Rev 32 (3). doi:10.1128/CMR.00001-19
2. Siu LK, Yeh KM, Lin JC, Fung CP, Chang FY (2012) Klebsiella pneumoniae liver abscess: a new invasive syndrome. Lancet Infect Dis 12 (11):881-887. doi:10.1016/S1473-3099(12)70205-0
3. Joob B, Wiwanitkit V (2017) Klebsiella pneumoniae Invasive Liver Abscess Syndrome and Endophthalmitis. J Emerg Med 53 (6):917. doi:10.1016/j.jemermed.2017.03.050
4. 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 (3):284-293. doi:10.1086/519262
5. Melot B, Brisse S, Breurec S, Passet V, Malpote E, Lamaury I, Thiery G, Hoen B (2016) Community-acquired meningitis caused by a CG86 hypervirulent Klebsiella pneumoniae strain: first case report in the Caribbean. BMC Infect Dis 16 (1):736. doi:10.1186/s12879-016-2065-2
6. Harada S, Doi Y (2018) Hypervirulent Klebsiella pneumoniae: a Call for Consensus Definition and International Collaboration. J Clin Microbiol 56 (9). doi:10.1128/JCM.00959-18
7. Lee IR, Molton JS, Wyres KL, Gorrie C, Wong J, Hoh CH, Teo J, Kalimuddin S, Lye DC, Archuleta S, Holt KE, Gan YH (2016) Differential host susceptibility and bacterial virulence factors driving Klebsiella liver abscess in an ethnically diverse population. Sci Rep 6:29316. doi:10.1038/srep29316
8. Struve C, Roe CC, Stegger M, Stahlhut SG, Hansen DS, Engelthaler DM, Andersen PS, Driebe EM, Keim P, Krogfelt KA (2015) Mapping the Evolution of Hypervirulent Klebsiella pneumoniae. mBio 6 (4):e00630. doi:10.1128/mBio.00630-15
9. Zhu J, Wang T, Chen L, Du H (2021) Virulence Factors in Hypervirulent Klebsiella pneumoniae. Front Microbiol 12:642484. doi:10.3389/fmicb.2021.642484
10. Peirano G, Pitout JD, Laupland KB, Meatherall B, Gregson DB (2013) Population-based surveillance for hypermucoviscosity Klebsiella pneumoniae causing community-acquired bacteremia in Calgary, Alberta. Can J Infect Dis Med Microbiol 24 (3):e61-64. doi:10.1155/2013/828741
11. Chou A, Nuila RE, Franco LM, Stager CE, Atmar RL, Zechiedrich L (2016) Prevalence of hypervirulent Klebsiella pneumoniae-associated genes rmpA and magA in two tertiary hospitals in Houston, TX, USA. J Med Microbiol 65 (9):1047-1048. doi:10.1099/jmm.0.000309
12. Cubero M, Grau I, Tubau F, Pallares R, Dominguez MA, Linares J, Ardanuy C (2016) Hypervirulent Klebsiella pneumoniae clones causing bacteraemia in adults in a teaching hospital in Barcelona, Spain (2007-2013). Clin Microbiol Infect 22 (2):154-160. doi:10.1016/j.cmi.2015.09.025
13. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. (2019).
14. Kumabe A, Kenzaka T (2014) String test of hypervirulent Klebsiella pneumonia. QJM 107 (12):1053. doi:10.1093/qjmed/hcu124
15. Lu J, Salzberg SL (2020) Ultrafast and accurate 16S rRNA microbial community analysis using Kraken 2. Microbiome 8 (1):124. doi:10.1186/s40168-020-00900-2
16. Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard AS, Garin B, Le Hello S, Arlet G, Nicolas-Chanoine MH, Decre D, Brisse S (2014) Genomic definition of hypervirulent and multidrug-resistant Klebsiella pneumoniae clonal groups. Emerg Infect Dis 20 (11):1812-1820. doi:10.3201/eid2011.140206
17. Chen YT, Chang HY, Lai YC, Pan CC, Tsai SF, Peng HL (2004) Sequencing and analysis of the large virulence plasmid pLVPK of Klebsiella pneumoniae CG43. Gene 337:189-198. doi:10.1016/j.gene.2004.05.008
18. 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 (14):4492-4501. doi:10.1128/JB.00315-09
19. Lery LM, Frangeul L, Tomas A, Passet V, Almeida AS, Bialek-Davenet S, Barbe V, Bengoechea JA, Sansonetti P, Brisse S, Tournebize R (2014) Comparative analysis of Klebsiella pneumoniae genomes identifies a phospholipase D family protein as a novel virulence factor. BMC Biol 12:41. doi:10.1186/1741-7007-12-41
20. Guo Y, Wang S, Zhan L, Jin Y, Duan J, Hao Z, Lv J, Qi X, Chen L, Kreiswirth BN, Wang L, Yu F (2017) Microbiological and Clinical Characteristics of Hypermucoviscous Klebsiella pneumoniae Isolates Associated with Invasive Infections in China. Front Cell Infect Microbiol 7:24. doi:10.3389/fcimb.2017.00024

21. Li L, Yuan Z, Chen D, Xie X, Zhang B (2020) Clinical and Microbiological Characteristics of Invasive and Hypervirulent Klebsiella pneumoniae Infections in a Teaching Hospital in China. Infect Drug Resist 13:4395-4403. doi:10.2147/IDR.S282982
22. Lin JC, Siu LK, Fung CP, Tsou HH, Wang JJ, Chen CT, Wang SC, Chang FY (2006) Impaired phagocytosis of capsular serotypes K1 or K2 Klebsiella pneumoniae in type 2 diabetes mellitus patients with poor glycemic control. J Clin Endocrinol Metab 91 (8):3084-3087. doi:10.1210/jc.2005-2749
23. Fazili T, Sharngoe C, Endy T, Kiska D, Javaid W, Polhemus M (2016) Klebsiella pneumoniae Liver Abscess: An Emerging Disease. Am J Med Sci 351 (3):297-304. doi:10.1016/j.amjms.2015.12.018
24. Parrott AM, Shi J, Aaron J, Green DA, Whittier S, Wu F (2021) Detection of multiple hypervirulent Klebsiella pneumoniae strains in a New York City hospital through screening of virulence genes. Clin Microbiol Infect 27 (4):583-589. doi:10.1016/j.cmi.2020.05.012
25. Lam MMC, Wyres KL, Judd LM, Wick RR, Jenney A, Brisse S, Holt KE (2018) Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in Klebsiella pneumoniae. Genome Med 10 (1):77. doi:10.1186/s13073-018-0587-5
26. Wyres KL, Lam MMC, Holt KE (2020) Population genomics of Klebsiella pneumoniae. Nat Rev Microbiol 18 (6):344-359. doi:10.1038/s41579-019-0315-1
27. Catalan-Najera JC, Garza-Ramos U, Barrios-Camacho H (2017) Hypervirulence and hypermucoviscosity: Two different but complementary Klebsiella spp. phenotypes? Virulence 8 (7):1111-1123. doi:10.1080/21505594.2017.1317412
28. Rodrigues C, d'Humieres C, Papin G, Passet V, Ruppe E, Brisse S (2020) Community-acquired infection caused by the uncommon hypervirulent Klebsiella pneumoniae ST66-K2 lineage. Microb Genom 6 (8). doi:10.1099/mgen.0.000419
29. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, Chan EW, Shu L, Yu J, Zhang R, Chen S (2018) A fatal outbreak of ST11 carbapenem-resistant hypervirulent Klebsiella pneumoniae in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis 18 (1):37-46. doi:10.1016/S1473-3099(17)30489-9
30. Emergence of hypervirulent Klebsiella pneumoniae ST23 carrying carbapenemase genes in EU/EEA countries (2021). European Centre for Disease Prevention and Control,
31. Russo TA, Olson R, Fang CT, Stoesser N, Miller M, MacDonald U, Hutson A, Barker JH, La Hoz RM, Johnson JR (2018) Identification of Biomarkers for Differentiation of Hypervirulent Klebsiella pneumoniae from Classical K. pneumoniae. J Clin Microbiol 56 (9). doi:10.1128/JCM.00776-18

Isolates	MSLT	scgMLST	Capsule	Sample Year	Age	Gender	Birth place	Hospital/ Province	Underlying condition	Anatomical Site	Primary Infection	Bacteriemia	Metastasis	ICU	Treatment	Outcome
S1	ST23	scgST-26	K1	2014	71	M	Belgium	H1/Antwerp	Benign prostatic hyperplasia	Blood	Liver abscess	+	-	-	FQ	Cured
S2	ST23	scgST-26	K1	2014	NR	M	DR Congo	H2/Brussels	HIV	Blood	Liver abscess	+	-	+	TZP, C3G	Death
S3	ST2039	scgST-441	K2	2014	81	M	Belgium	H3/Brussels	Cholecystis	Hepato/biliary	Liver abscess	-	-	+	AMC / SI	Cured
S4	ST23	scgST-26	K1	2014	62	M	DR Congo	H4/Brussels	HIV / Diabetes	Blood	Liver abscess	+	Endophtalmitis	+	AMC, FQ / SI	Cured
S5	ST2039	scgST-441	K2	2015	57	F	Belgium	H5/Namur	Oropharyngeal carcinoma	Blood	Pneumoniae	+	-	+	AMC, TZP	Death
S6	ST23	scgST-26	K1	2016	68	M	Belgium	H2/Brussels	Colorectal adenocarcinoma	Ocular	Endophtalmitis	+	Liver / Brain abcess	-	MEM, C3G	Cured
S7	ST86	scgST-47	K2	2016	27	M	Cameroon	H4/Brussels	Diabetes	Hepato/biliary	Liver abscess	+	-	-	C3G, FQ / SI	Cured
S8	ST380	scgST-53	K2	2016	52	M	Morocco	H4/Brussels	HIV / HCV / Alcoholism	Blood	Pulmonary abscess	+	-	+	TZP, FQ	Death
S9	ST65	scgST-283	K2	2016	81	F	Belgium	H2/Brussels	-	Blood	Liver abscess	+	-	+	C3G, FQ / SI	Cured
S10	ST2039	scgST-441	K2	2016	80	M	Belgium	H5/Namur	Diabetes / COPD / Prostatectomy	Testicle	Orchiepididymitis	+	Lung	-	C4G, TZP, SXT, C1G / SI	Death
S11	ST23	scgST-26	K1	2017	73	F	NR	H2/Brussels	NR	Blood	Liver abscess	+	NR	-	NR	NR
S12	ST380	scgST-53	K2	2017	72	M	Belgium	H3/Brussels	Diabetes / Colorectal Adenocarcinoma	Blood	Liver abscess	+	-	-	AMC, MEM, FQ / SI	Cured
S13	ST66	scgST-419	K2	2018	44	M	NR	H6/East Flanders	NR	Blood	Pulmonary abscess	+	NR	-	NR	NR
S14	ST65	scgST-283	K2	2018	47	M	Morocco	H7/Brussels	-	Blood	Liver abscess	+	-	-	TZP, FQ, C3G, SXT	Cured
S15	ST25	scgST-1533	K2	2019	39	M	Romania	H2/Brussels	Alcoholic steatohepatitis	Blood	Pulmonary abscess	+	-	+	MEM, C3G	Death
S16	ST23	scgST-26	K1	2019	75	F	Belgium	H1/Antwerp	Previous liver abscess / Alcoholism	Blood	Liver abscess	+	-	+	FQ	Cured
S17	ST3240	scgST-55	K2	2019	60	M	Morocco	H5/Namur	Diabetes / Partial Hepatectomy	Hepato/biliary	Liver abscess	+	-	-	AMC, C3G, FQ / SI	Cured
S18	ST23	scgST-26	K1	2019	67	M	China	H1/Antwerpen	Diabetes / Chronic myeloid leukemia	Hepato/biliary	Liver abscess	+	-	+	TZP, FQ	Cured
S19	ST380	scgST-53	K2	2019	67	M	Belgium	H5/Namur	Benign prostatic hyperplasia / Cholecystis	Blood	Liver abscess	+	-	-	TZP, AMC, C3G, FQ, MEM / SI	Cured
S20	ST592	scgST-547	K57	2019	46	M	Guinea-Bissau	H8/Brussels	Diabetes / HBV / Hepatic Cirrhosis	Muscle	Necrotizing fasciitis	+	-	+	SI	Death
S21	ST65	scgST-283	K2	2019	55	M	NR	H8/Brussels	-	Blood	Liver abscess	+	-	-	AMC	Cured
S22	ST66	scgST-419	K2	2020	63	F	Belgium	H5/Namur	Alcoholism	Blood	Pulmonary abscess	+	-	-	TZP, FQ, C1G / SI	Cured

Table 1: Clinical characteristics of hypervirulent *K. pneumoniae* isolates

NR: Not reported; ICU: Intensive care unit admission; HIV: Human immunodeficiency virus; HCV: hepatitis C virus; COPD: Chronic obstructive pulmonary disease; HBV: hepatitis B virus; SI: Drainage or surgical intervention; FQ: Fluoroquinolone; TZP: Piperacillin/tazobactam; AMC: Amoxicillin/clavulanic acid; C1G: First-generation cephalosporin; C3G: Third-generation cephalosporin; C4G: Fourth-generation cephalosporin; MEM: Meropenem; SXT: Trimethoprim/Sulfamethoxazole

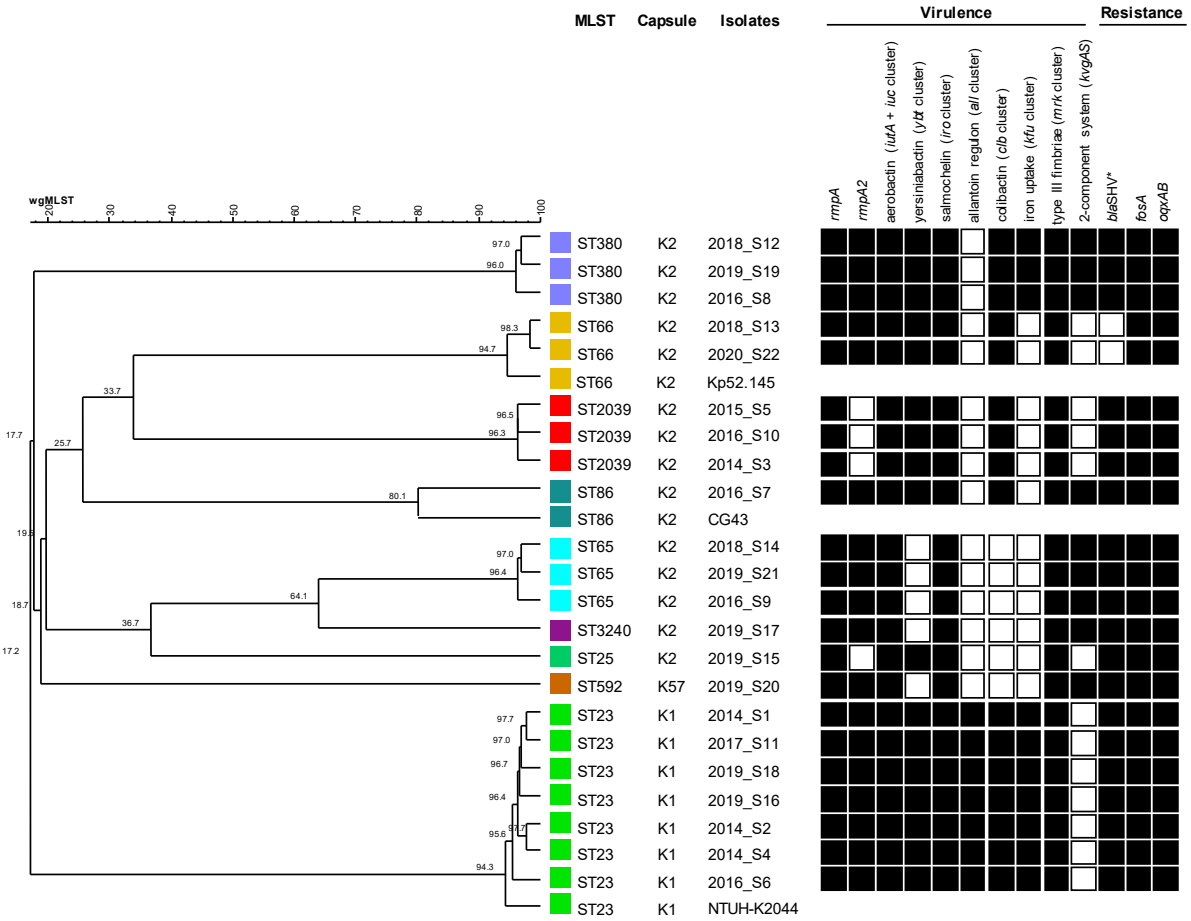

Figure 1 : Clonal relationship of the hypervirulent *K. pneumoniae* isolates based on the whole-genome multilocus and distribution of virulence and resistance features. The wgMLST phylogenic tree was generated with Bionumerics 8.0 using categorical values with a scaling factor of 100 (100*number of the loci in common/total number of loci in the complete comparison). The three hyperviruent *K. pneumoniae* reference strains CG43, NTUH-K2044, Kp52.145 were included in the analysis. MLST (ST) and capsular type of each isolate are indicated on the left of the dendrogram. A black square indicates presence of the corresponding gene. *$bla_{SHV-190}$ for ST23; $bla_{SHV-110}$ for ST25; bla_{SHV-11} for ST65; bla_{SHV-28} for ST86; bla_{SHV-33} for ST380; bla_{SHV-26} for ST592; bla_{SHV-40} for ST2039; bla_{SHV-99} for ST3240