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Short communication

The emergence of hypervirulent *bla*$_{NDM-1}$-positive *Klebsiella pneumoniae* sequence type 395 in an oncology hospital

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ABSTRACT

Fifteen hypermucoviscous isolates (13 *bla*$_{NDM-1}$-positive) obtained from 11 oncology patients were analyzed by whole genome sequencing, and selected isolates were assessed in a murine model of sepsis. ST395/K2 isolates harboring *rmpA*, *rmpA2*, *peg-344*, aerobactin, enterobactin, yersiniabactin, type I fimbriae, etc. displayed maximal virulence in the mouse lethality assay (LD_{50} = 10^2 CFU). ST147/K20 isolates lacking yersiniabactins were relatively less virulent (LD_{50} = 10^4 CFU), ST395/K2 isolates lacking *rmpA*, *rmpA2*, p*eg-344*, and aerobactin, but harboring yersiniabactin demonstrated minimal virulence (LD_{50} = 10^5 CFU). Isolates represent various paths and stages of evolution directed towards convergence of multidrugresistant classical *Klebsiella pneumoniae* and hypervirulent *K. pneumoniae*.

1. Introduction

Klebsiella pneumoniae is a causative agent of community- and hospital-acquired infections worldwide and has two pathotypes. Classical *K. pneumoniae* (cKp) are predominant, causing infections primarily in patients with comorbidities or in immunocompromised patients and have acquired numerous resistance genes including KPC-, OXA-48-, and NDM-type carbapenemases (Navon-Venezia et al., ***2017***). Hypervirulent *K. pneumoniae* (hvKp) initially were considered to cause community-acquired pyogenic liver abscesses in healthy individuals, hypermucoviscosity being the primary phenotype, and isolates were usually susceptible to antimicrobial agents (Shon et al., ***2013***). Subsequently, it was clarified that hypermucoviscosity is often not correlated with hypervirulence. Increased capsular production and plasmid-born genes *peg-344*, *iroB*, *iucA*, *prmpA*, and *rmpA2*, are genetic markers of hypervirulent phenotype (Russo et al., ***2018***). The role of capsular types in virulence is not fully understood. K1 or K2 capsular types represent most hvKp isolates, but other types (K5, K16, K20, K54, and K57) (Shon et al., ***2013***) have been described as virulent also.

For long time hvKp and multidrug-resistant (MDR) cKp lineages were non-overlapping, however convergence of virulence and resistance is the most unfavorable trend (Gu et al., ***2018***; Zhang et al., ***2019***). Recently, hypermucoviscous carbapenem-resistance *K. pneumoniae* isolates were sporadically recovered in an oncology hospital in St. Petersburg. This study aimed to characterize hypermucoviscous isolates at the molecular level and to evaluate their virulence.

2. Methods

From April 2017 to December 2018, all hypermucoviscous *K. pneumoniae* isolated in a hospital laboratory as part of routine patient care were included in the study. Isolates were identified via matrix-assisted laser desorption/ionization mass spectrometry using a Microflex LT (Bruker Daltonics ltd., Germany), and the hypermucoviscous phenotype was detected via the string test (Hadano, 2013). Minimum inhibitory concentrations (MICs) of antibiotics of main

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groups were determined via broth microdilution and interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (*http://www.eucast.org/clinical_breakpoints*). The isolates were characterized via Illumina MiSeq sequencing (Nextera XT libraries, paired-end 300-bp reads), and reads were assembled de novo into contigs by SPAdes algorithm v.3.10.0 (GenBank BioProject accession number **PRJNA522420)**. Phylogenetic analysis based on core-SNP alignment was performed using kSNP3.0 software under default settings (Gardner et al., *2015*). The isolates were screened for antimicrobial resistance and virulence genes and plasmids with ABRicate (*https://github.com/tseemann/abricate*). Capsule types were determined using Kaptive (Wyres et al., *2016*); and sequence types (STs) - using MLST 2.0 (Center for Genomic Epidemiology; *http://www.genomicepidemiology.org*). The virulence of selected isolates was assessed in a mouse lethality assay and LD_{50} values were determined using the (Ryyв and Muench, *1938*) method (Supplementary material, Text S1).

3. Results and discussion

Fifteen hypermucoviscous *K. pneumoniae* isolates from 11 patients were analyzed. The characteristics of patients and isolates, including five virulence genes considered to promote cKp and hvKp differentiation (Russo et al., *2018*), resistance profiles, resistance genes, and plasmid replicons are enlisted in Table 1. MIC values of antibiotics of main groups are presented in Supplementary material (Table S1). All patients had multiple comorbidities and solid tumors at different locations. Most isolates were obtained from sites not associated with invasive diseases. In one patient (№ 7), three isolates with different properties were recovered, and two different isolates were recovered from two patients (№ 2 and 10).

The estimated average coverage across the assembled genomes of studied isolates varied from 33 to 106, number of contigs varied from 71 to 88 with an N50 scaffold length varied from 245,962 to 709,400. *K. pneumoniae* isolates belonged to two STs: ST395 (n = 9) and ST147 (n = 6). The isolates of each sequence type were closely related, differences according core-SNP phylogenetic analysis do not exceed 20 SNPs.

Five groups of hypermucoviscous isolates were distinguished based on the presence of virulence genes and survival of mice in lethality assay. The first group consisted of five ST395/K2 bla_{NDM-1} positive isolates, which harbored similar but unidentical sets of virulence genes: *rmpA*, *rmpA2*, and *peg-344*; aerobactin, enterobactin, and yersiniabactin clusters; *fimA* and *fimE* genes, and others. The differences between the isolates of the group were minimal: three of five isolates lacked *terB* gene, and isolate 2021 lacked *rmpA2* gene. The 1657 isolate (representative of the group) was recovered from a deceased patient and was highly virulent in the mouse lethality assay (LD_{50} = 10^2 CFU). The second group included two other ST395/K2 isolates, which, unlike the previous group, lacked *rmpA* gene, and were significantly less virulent in the mouse lethality assay (LD_{50} of representative isolate 1971 = 10^5 CFU). Isolates of both groups harbored different additional beta-lactamases ($bla_{CTX-M-15}$, bla_{OXA-1} bla_{OXA-9}, bla_{TEM-1B} and $bla_{SHV-182}$), and other resistance genes. All isolates carried four plasmid replicons (IncFIB(Mar), IncHI1B, IncR, and ColRNAI). Three additional replicons (IncFIB(pQil), IncFII(pRSB107), and IncFII were detected in two isolates from group 1, and in one isolate of group 2.

The third and fourth groups were represented by single bla_{NDM-1}-negative ST395/K2 and ST395/K108 isolates respectively. The isolates were included in the study as possible donors of virulence genes; however, despite hypermucoviscous phenotype the strains displayed a low virulence phenotype. ST395/K2 isolate lacked *rmpA2* and aerobactin, but carried *peg-344*, it demonstrated low virulence in the mouse lethality assay (LD_{50} = 10^5 CFU). ST395/K108 lacked *rmpA*, *rmpA2*, *peg-344*, aerobactin and yersiniabactin clusters; it was avirulent (LD_{50} > 10^6 CFU).

K. pneumoniae ST395 isolates were genetically highly similar and probably epidemiologically linked, although we have no evidence of direct contact between patients, and the time interval between the isolation of individual strains ranged from three weeks to ten months. Two isolates obtained from the patient № 10 were the exceptions. Isolate 2024 was recovered from sputum on August 01, 2018, and did not contain genes of aerobactin cluster and bla_{NDM}. Isolate 2021 ***harboring*** aerobactin cluster, bla_{NDM}, and three additional plasmid replicons was recovered five days later from urine. Genomes of 2024 and 2021 isolates differed by only eight SNP and it was impossible to differentiate between reinfection with closely related strain and acquisition of resistance and virulence genes in vivo.

The fifth group consists of six ST147/K20 isolates, which harbored identical sets of virulence genes: *rmpA*, *rmpA2*, and *peg-344*, aerobactin and enterobactin clusters, and other genes, the absence of yersiniabactin cluster was a common feature of the group. Isolate 1659 (representative of the group) demonstrated moderate virulence in the mouse lethality assay (LD_{50} = 10^4 CFU). Isolates of this group were highly homogeneous; they demonstrated practically identical profiles of resistance, harbor identical sets of corresponding resistance genes, and five plasmid replicons (IncFIB(Mar), IncHI1B, IncR, ColRNAI, and IncFIB(pKPHS1)).

Differences in virulence of groups representative isolates are illustrated at Kaplan–Meier survival plots of mice infected with prototypical isolates (Supplementary material, Fig. S1).

ST147 $bla_{NDM-type}$ positive *K. pneumoniae* are common worldwide (Wu et al., *2019*), however they were not previously reported from St. Petersburg, where ST340 and ST395 are predominant (Ageevets et al., *2014*). ST147 $bla_{NDM-type}$ positive isolate harboring a number of virulence genes (the aerobactin cluster, *rmpA/rmpA2*, and some others) located on a large plasmid was reported from UK (Turton et al., *2018*). *K. pneumoniae* ST395 unlike ST147 is not recognized as high-risk clone (Wyres et al., *2020*), and to our knowledge, it is the first description of bla_{NDM-1}-positive ST395/K2 hvKp isolates. As of December 2019 among 7245 complete and draft *K. pneumoniae* genomes in GenBank 46 belonged to ST395, 17 of them were $bla_{NDM-type}$ positive, and only one harbored *rmpA* gene (Supplementary material, Table S2).

It was impossible to obtain complete plasmids from short-read sequencing however, indirect data indicate the possible role of *K. pneumoniae* ST147, carrying plasmids similar to those described in the UK, as a donor of virulence genes for $bla_{NDM-type}$ positive ST395 in our hospital. ST147 isolates harbored 118–163 kpb contigs containing *peg-344*, *rmpA*, *rmpA2* and aerobactin cluster genes. Genomes of ST395/K2 isolates contained similar 26–160 kbp contigs ***harboring*** *peg-344*, and aerobactin cluster genes, some of them carried *rmpA*, and *rmpA2* genes. The contigs demonstrating at least 99% coverage and at least 99.98% identity with plasmid pKpvST147B_virulence from UK isolate KpvST147B (Turton et al., *2019*). Acquisition of virulence genes by resistant lineages of *K. pneumoniae* is more probable than acquisition of resistance by virulent clones (Wyres et al., *2019*). However, emergence of mosaic plasmids carrying both virulence and resistance determinants (Lam et al., *2019*) and acquisition of resistance plasmids by hypervirulent lineages have also been described (Chen et al., *2020*). ST147/K20 and ST395/K2 bla_{NDM-1}-positive isolates represent various paths and stages of evolution directed towards convergence of hvKp and multidrugresistant cKp and emergence of new high-risk clone.

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Ethical approval

Ethics approval was obtained from the Clinical Research and Practical Center of Specialized Types of Medical Care (Oncologic)

Table 1
Characteristics of patients and Klebsiella pneumoniae isolates included in the study.

Traits		Genes	Sequence types/K-types, groups numbers, isolates numbers														
ST			ST395									ST147					
K-type			K2								K108	K20					
Group			1					2		3	4	5					
Isolate №			1657	1983	2021	2108	1966	1961	1971	2024	1658	1659	1968	2026	1976	2036	2032
Patient №			2	7	10	11	6	3	4	10	1	2	5	8	7	7	9
Source of isolates[a]			W	W	Ur	Ur	B	BAL	W	S	W	Uc	W	PF	Ur	PF	PF
Outcome[b]			D	D	U	U	U	D	U	U	U	D	D	D	HG	U	D
Mouse lethality (LD_{50}, CFU)			10^2	Nt[c]	Nt	Nt	Nt	Nt	10^5	10^5	$> 10^6$	10^4	Nt	Nt	Nt	Nt	Nt
Virulence genes	Salmochelin	*iroB*[d]	-[e]	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Regulators of mucoid	*rmpA*[d]	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+
	phenotype	*rmpA2*[d]	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+
	Transporter	*peg344*[d]	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
	Aerobactin cluster	*iucA*[d], *iucB*, *iucC*, *iucD*, *iutA*	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+
	Enterobactin cluster	*entB*, *entC*, *fepC*, *fepG*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Yersiniabactin cluster	*fyuA*, *ipr1*, *ipr2*, *ybtA*, *ybtE*, *ybtP*, *ybtQ*, *ybtS*, *ybtT*, *ybtU*, *ybtX*	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	Fimbria	*fimA*, *fimB*, *fimE*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Other	*yagV/ecpE*, *yagW/ecpD*, *yagX/ecpC*, *yagY/ecpB*, *ykgK/ecpR*, *yagZ/ecpA*, *mgtC*, *ompA*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		terB	+	−	−	+	−	+	+	−	+	+	+	+	+	+	+

(*continued on next page*)

Table 1 (*continued*)

Traits		Genes	Sequence types/K-types, groups numbers, isolates numbers														
ST			ST395									ST147					
K-type			K2								K108	K20					
Group			1					2		3	4	5					
Isolate №			1657	1983	2021	2108	1966	1961	1971	2024	1658	1659	1968	2026	1976	2036	2032
Patient №			2	7	10	11	6	3	4	10	1	2	5	8	7	7	9
Source of isolates[a]			W	W	Ur	Ur	B	BAL	W	S	W	Uc	W	PF	Ur	PF	PF
Outcome[b]			D	D	U	U	U	D	U	U	U	D	D	D	HG	U	D
Mouse lethality (LD_{50}, CFU)			10^2	Nt[c]	Nt	Nt	Nt	Nt	10^5	10^5	$> 10^6$	10^4	Nt	Nt	Nt	Nt	Nt
Resistance genes	Aminoglycosides	*aadA1*	−	−	+	+	−	−	−	−	+	+	+	+	+	+	+
		aph(3′)-VI	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+
		aph(3′)-Ia	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−
		aac(6′)-Ib-cr	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−
		aac(6′)-Ib	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+
		armA	−	−	+	−	−	+	+	−	−	+	+	+	+	+	+
		aac(3)-IIa	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
		Resistance profiles[f]	−	Am	Gn, Am	Am	Gn, Am	Gn, Am		−	Gn	Gn	Gn, Am	Am	Gn, Am	Gn, Am	Gn, Am
	Beta-lactams	bla_{NDM-1}	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+
		$bla_{CTX-M-15}$	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		bla_{OXA-1}	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−
		bla_{OXA-9}	−	−	+	+	−	−	−	−	−	+	+	+	+	+	+
		bla_{TEM-1A}	−	−	−	−	−	+	−	−	−	+	+	+	+	+	+
		bla_{TEM-1B}	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−
		bla_{SHV-67}	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+
		$bla_{SHV-182}$	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
		Resistance profiles[f]	Cf, Ip, Mp, Ca					Cf, Ip, Mp, Ca			Cf	Cf	Cf, Ip, Mp, Ca				
	Fluoroquinolones	*aac(6′)-Ib-cr*	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+
		oqxB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		qnrS1	+	+	+	+	+	+	+	+	−	−	−	+	−	−	+
		qnrS2	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
		oqxA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Resistance profiles[f]	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci	Ci
	Fosfomycin	*fosA*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Resistance profiles[f]	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs	Fs
	MLS	*msr(E)*	−	−	+	−	−	+	+	−	−	+	+	+	+	+	+
		mph(E)	−	−	−	−	−	+	+	−	−	+	+	+	+	+	+
		mph(A)	+	+	+	+	+	+	+	−	−	+	−	+	+	+	+
	Sulphonamides	*sul1*	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+
		sul2	−	−	−	−	−	+	+	−	−	+	+	+	+	+	+
	Trimetoprim	*dfrA1*	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−
		dfrA5	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+
		Resistance profiles[f]	Sx	Sx	Sx	Sx	Sx	Sx	Sx	Sx	−	Sx	Sx	Sx	Sx	Sx	Sx
	Phenicols	*catB*	−	catB3	−	−	catB3	−	−	catB7	−	catB3	−	catB3	−	catB3	−
		catA	catA1	catA1	−	−	catA1	−	−	−	−	catA1	−	catA1	−	catA1	−
	Tetracyclines	*tet(A)*	+	−	+	+	−	−	−	−	−	−	−	+	−	−	+
		Resistance profiles[f]	−	−	−	Tg	−	−	−	−	−	−	−	Tg	Tg	−	−

(*continued on next page*)

Table 1 (*continued*)

Traits	Genes	Sequence types/K-types, groups numbers, isolates numbers														
ST		ST395									ST147					
K-type		K2								K108	K20					
Group		1					2		3	4	5					
Isolate №		1657	1983	2021	2108	1966	1961	1971	2024	1658	1659	1968	2026	1976	2036	2032
Patient №		2	7	10	11	6	3	4	10	1	2	5	8	7	7	9
Source of isolates[a]		W	W	Ur	Ur	B	BAL	W	S	W	Uc	W	PF	Ur	PF	PF
Outcome[b]		D	D	U	U	U	D	U	U	U	D	D	D	HG	U	D
Mouse lethality (LD_{50}, CFU)		10^2	Nt[c]	Nt	Nt	Nt	Nt	10^5	10^5	$> 10^6$	10^4	Nt	Nt	Nt	Nt	Nt
Plasmid replicons	IncFIB(Mar)	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
	IncHI1B	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
	IncR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ColRNAI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IncFIB(pQil)	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−
	IncFII(pRSB107)	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−
	IncFII	−	−	+	+	−	+	−	−	+	−	−	−	−	−	−
	IncFII(pHN7A8)	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
	IncFIB(K)	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
	ColpVC	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
	IncFIB(pKPHS1)	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+

[a] Source of isolates: W-wound; BAL - broncho-alveolar lavage; B - blood; Ur - urine; S - sputum; PF - peritoneal fluid; UC - urine catheter;
[b] Outcome: U - unaltered; D - death; HG - health gain;
[c] Nt – not tested;
[d] genes used for cKp and hvKp differentiation (Russo et al., ***2018***),
[e] (+) presence of the gene; (−) absence of the gene;
[f] Resistance profiles: MLS – macrolides, lincosamides and streptogramines; Cf – cefotaxime and ceftazidime; Gn – gentamicin; Am – amikacin; Ip – imipenem; Mp – meropenem; Sx – co-trimoxazole, Ci – ciprofloxacin; Ca – ceftazidime/avibactam, Fs – fosfomycin; Tg - tigecycline.

human research ethics committee (№ 90–2018). This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Declaration of Competing Interest

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2020.104527.

References

Ageevets, V.A., Partina, I.V., Lisitsyna, E.S., Ilina, E.N., Lobzin, Y.V., Shlyapnikov, S.A., Sidorenko, S.V., 2014. Emergence of carbapenemase-producing Gram-negative bacteria in Saint Petersburg, Russia. Int. J. Antimicrob. Agents 44, 152–155. https://doi.org/10.1016/j.ijantimicag.2014.05.004.

Chen, Y., Marimuthu, K., Teo, J., Venkatachalam, I., Cherng, B., De Wang, L., Prakki, S., Xu, W., Tan, Y.H., Nguyen, L.C., Koh, T.H., Ng, O.T., Gan, Y.-H., 2020. Acquisition of ***p***lasmid with Carbapenem-***r***esistance ***g***ene blaKPC2 in Hypervirulent Klebsiella pneumoniae, Singapore. Emerg. Infect. Dis. 26, 549–559. https://doi.org/10.3201/eid2603.191230.

Gardner, S.N., Slezak, T., Hall, B.G., 2015. kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. Bioinformatics 31, 2877–2878. https://doi.org/10.1093/bioinformatics/btv271.

Gu, D., Dong, N., Zheng, Z., Lin, D., Huang, M., Wang, L., Chan, E.W., 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, 37–46. https://doi.org/10.1016/S1473-3099(17)30489-9.

Lam, M.M.C., Wyres, K.L., Wick, R.R., Judd, L.M., Fostervold, A., Holt, K.E., Löhr, I.H., 2019. Convergence of virulence and MDR in a single plasmid vector in MDR Klebsiella pneumoniae ST15. J. Antimicrob. Chemother. 74, 1218–1222. https://doi.org/10.1093/jac/dkz028.

Navon-Venezia, S., Kondratyeva, K., Carattoli, A., 2017. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol. Rev. 41, 252–275. https://doi.org/10.1093/femsre/fux013.

Russo, T.A., Olson, R., Fang, C.T., Stoesser, N., Miller, M., MacDonald, U., Hutson, A., Barker, J.H., La Hoz, R.M., Johnson, J.R., 2018. Identification of ***b***iomarkers for ***d***ifferentiation of Hypervirulent Klebsiella pneumoniae from Classical K. pneumoniae. J. Clin. Microbiol. 56. https://doi.org/10.1128/JCM.00776-18.

Ryyв, L.J., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. Am. J. Epidemiol. 27, 493–497. https://doi.org/10.1093/oxfordjournals.aje.a118408.

Shon, A.S., Bajwa, R.P., Russo, T.A., 2013. Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: a new and dangerous breed. Virulence 4, 107–118. https://doi.org/10.4161/viru.22718.

Turton, J.F., Payne, Z., Coward, A., Hopkins, K.L., Turton, J.A., Doumith, M., Woodford, N., 2018. Virulence genes in isolates of Klebsiella pneumoniae from the UK during 2016, including among carbapenemase gene-positive hypervirulent K1-ST23 and 'non-hypervirulent' types ST147, ST15 and ST383. J Med Microbiol 67, 118–128. https://doi.org/10.1099/jmm.0.000653.

Turton, J., Davies, F., Turton, J., Perry, C., Payne, Z., Pike, R., 2019. Hybrid ***r***esistance and ***v***irulence ***p***lasmids in "***h***igh-***r***isk" ***c***lones of Klebsiella pneumoniae, ***i***ncluding ***t***hose ***c***arrying blaNDM-5. Microorganisms 7, 326. https://www.mdpi.com/2076-2607/7/9/326.

Wu, W., Feng, Y., Tang, G., Qiao, F., McNally, A., Zong, Z., 2019. NDM Metallo-β-***l***actamases and ***t***heir ***b***acterial ***p***roducers in ***h***ealth ***c***are ***s***ettings. Clin. Microbiol. Rev. 32, e00115–e00118. https://doi.org/10.1128/cmr.00115-18.

Wyres, K.L., Wick, R.R., Gorrie, C., Jenney, A., Follador, R., Thomson, N.R., Holt, K.E., 2016. Identification of Klebsiella capsule synthesis loci from whole genome data. Microbial Genomics 2. https://doi.org/10.1099/mgen.0.000102.

Wyres, K.L., Wick, R.R., Judd, L.M., Froumine, R., Tokolyi, A., Gorrie, C.L., Lam, M.M.C., Duchêne, S., Jenney, A., Holt, K.E., 2019. Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of Klebsiella pneumoniae. PLoS Genet. 15, e1008114. https://doi.org/10.1371/journal.pgen.1008114.

Wyres, K.L., Lam, M.M.C., Holt, K.E., 2020. Population genomics of Klebsiella pneumoniae. Nat. Rev. Microbiol. 18, 344–359. https://doi.org/10.1038/s41579-019-0315-1.

Zhang, Y., Jin, L., Ouyang, P., Wang, Q., Wang, R., Wang, J., Gao, H., Wang, X., Wang, H., Network, C.C.-R.E., 2019. Evolution of hypervirulence in carbapenem-resistant Klebsiella pneumoniae in China: a multicentre, molecular epidemiological analysis. J. Antimicrob. Chemother. 75, 327–336. https://doi.org/10.1093/jac/dkz446.