The contribution of capsule polysaccharide genes to virulence of *Klebsiella pneumoniae*

Kwan Soo Ko

Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea

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*K. pneumoniae* is a Gram-negative, non-motile bacterium that is found in the environment, including in soil and surface water. *K. pneumoniae* readily colonizes human mucosal surfaces, such as those found in the gastrointestinal tract and oropharynx. *K. pneumoniae* can spread from colonizing sites to other tissues, leading to urinary tract infections, pneumonia, and other infections in humans.\(^1\) Recently, concerns have arisen due to the emergence and dissemination of hypervirulent (HV) or antibiotic resistant strains.\(^2,3\) To date, several *K. pneumoniae* virulence factors have been identified: the capsule, lipopolysaccharide, siderophores, and fimbriae (also known as pili).\(^1\) Of these, the capsule, which is synthesized by gene products from the capsular polysaccharide synthesis (*cps*) locus, is the most thoroughly studied virulence factor for *K. pneumoniae*. In the bacterium, the capsule confers resistance against the bactericidal activity of antimicrobial peptides, complement, and phagocytes.\(^1\) In addition, the capsule averts fulminant activation of the immune response.\(^4\) Acapsular *K. pneumoniae* strains are markedly less virulent than isogenic encapsulated strains in mouse models.\(^5,6\) and the strains that produce a capsule conferring a hypermucoviscous phenotype become HV.\(^7\) Over 70 capsule serotypes have been reported for *K. pneumoniae*.\(^8\) Among these, strains with the K1 and K2 capsule serotypes, which mainly cause liver abscess and belong to particular clones,\(^9,10\) are known to be hypermucoviscous or HV.\(^7,11\) In addition to K1 and K2, other serotypes have been described as HV, including K5, K16, K20, K54, and K57.\(^2\) Although it is known that certain serotypes are more closely associated with human colonization or infection, the particular factors conferring their increased virulence are currently unclear.

In this issue of *Virulence*, Lin et al.\(^12\) describe the contribution of genes located in the highly conserved region of the *cps* locus to the virulence of *K. pneumoniae*. In this study, Lin et al. identified a serotype K20 strain, which was isolated from a patient with a liver abscess, with high lethality in mice. The K20 strain belonged to ST268 based on multi-locus sequence typing analysis. Although the strain carried chromosomal and plasmid regulators of mucoid phenotype, *c-rmpA*, *p-rmpA*, and *p-rmpP*, and several virulence determinants including aerobactin (*iucA*), yersiniabactin (*irp2*), salmochelin (*iroB*), enterobactin (*entB*), and iron-uptake system (*kfu*), it did not show a hypermucoviscous phenotype. The authors deleted 6 conserved genes (*galF, acidPPC, wzi, wza, wzb, and wzc*) from the *cps* locus of the K20 strain, and generated recombinants by complementation of the deleted mutants with genes from the K20 or K1 strains. They then characterized these mutations’ effects on the bacterial virulence with respect to neutrophil phagocytosis, serum resistance, serum agglutination, and 50% lethal dose (LD\(_{50}\)) in mice.

Lin et al. determined that the 6 homologous genes from the *cps* locus could be categorized based on their effects on virulence.\(^12\) While deletion of *galF* and *acidPPC* genes, which are driven by the P1 promoter,\(^13\) had limited effect on virulence, the deletion of genes driven by the P2 promoter (*wzi, wza, wzb, and wzc*) exhibited moderate to high effects on virulence. The deletion mutants of the latter genes, which are responsible for surface assembly, CPS polymerization, and CPS production, also showed high susceptibility to neutrophilic phagocytosis and to serum. Thus, it appears that the homologous genes’ influence on virulence differs according to their associated promoter or role in CPS biosynthesis. In contrast, creation of the *cps* gene recombinant mutants for the K1 or K20 strains did not impart consistent results. Recombinant mutants with the...
acidPPC gene from the K1 strain restored the virulence of the wild-type K20 strain in regards to serum killing, phagocytosis, and lethality in mice. However, mutants with wzi, wza, wzb, and wzc from the K1 strain exhibited inconsistent results in lethality, phagocytosis, and serum killing. For example, the recombinant with wzi of the K1 strain restored phagocytosis, but not serum killing and lethality, while the wza recombinant improved phagocytosis and serum resistance, but not lethality. Based on these observations, the authors conclude that the homologous genes for capsule biosynthesis in different serotypes of *K. pneumoniae* do not function in the same manner after gene switching.

This study by Lin et al. is significant for its characterization of the various effects that can be attributed to the homologous genes from different *K. pneumoniae* serotypes. However, the study was limited in that it was based on a single strain of each serotype, and thus the relevance of this study’s results in regards to other *K. pneumoniae* strains and serotypes must be explored. In addition, many studies indicate an association between serotype and other features, and between production of CPS and antibiotic resistance. Further studies, including serotype switching, are warranted in order to more fully understand the effects of the K antigen or serotype on virulence and other features.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

### References

1. Paczova MK, Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiol Mol Biol Rev 2016; 80: 629-61; PMID:27307579; https://doi.org/10.1128/MMBR.00078-15

2. Shorn AS, Bajwa RPS, Russo TA. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: A new and dangerous breed. Virulence 2013; 4:107-18; PMID:23307290; https://doi.org/10.4161/viru.22718

3. Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 2011; 35:736-55; PMID:21303394; https://doi.org/10.1111/j.1574-6976.2011.02268.x

4. Li B, Zhao Y, Liu C, Chen Z, Zhou D. Molecular pathogenesis of *Klebsiella pneumoniae*. Future Microbiol 2014; 9:1071-81; PMID:25340836; https://doi.org/10.2217/fmb.14.48

5. Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Yamaguchi K. Role of bacterial capsule in local and systemic inflammatory responses of mice during pulmonary infection with *Klebsiella pneumoniae*. J Med Microbiol 2000; 49:1003-10; PMID:11073154; https://doi.org/10.1099/0022-1317-49-11-1003

6. Lawlor MS, Hsu J, Rick PD, Miller VL. Identification of *Klebsiella pneumoniae* virulence determinants using an intranasal infection model. Mol Microbiol 2005; 58:1054-73; PMID:16262790; https://doi.org/10.1111/j.1365-2958.2005.04918.x

7. Fang CT, Lai SY, Yi WC, Hsueh PR, Liu KL, Chang SC. *Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin Infect Dis 2007; 45:284-93; PMID:17599305; https://doi.org/10.1086/519262

8. Pan YJ, Fang HC, Yang HC, Lin TL, Hsieh PF, Tsai FC, Keynan Y, Wang JT. Capsular polysaccharide synthesis regions in *Klebsiella pneumoniae* serotype K57 and a new capsular serotype. J Clin Microbiol 2008; 46:2231-40; PMID:18508935; https://doi.org/10.1128/JCM.00716-07

9. Chung Dr, Park MH, Kim SH, Ko KS, Kang CL, Peck KR, Song JH. Prevalence and molecular characterization of serotype K1 *Klebsiella pneumoniae* strains from various clinical specimen sources in 11 Asian countries. J Infect 2012; 64:622-5; PMID:22343125; https://doi.org/10.1016/j.jinf.2012.02.007

10. Struve C, Roe CC, Stegger M, Stahlhut SG, Hansen DS, Engerhalder DM, Andersen PS, Driebe EM, Keim P, Krogfelt KA. Mapping the evolution of hypervirulent *Klebsiella pneumoniae*. mBio 2015; 6:e00630-15; https://doi.org/10.1128/mBio.00630-15

11. Yeh KM, Chiu SK, Lin CL, Huang LY, Tsai YK, Chang JC, Lin JC, Chang FY, Siu LK. Surface antigens contribute differently to the pathophysiological features in serotype K1 and K2 *Klebsiella pneumoniae* strains isolated from liver abscesses. Gut Pathog 2016; 8:4; PMID:26893615; https://doi.org/10.1186/s13099-016-0085-5

12. Lin CL, Chen FH, Huang LY, Chang JC, Chen JH, Tsai YK, Chang FY, Lin JC, Siu LK. Effect in virulence of switching conserved homologous capsular polysaccharide genes from *Klebsiella pneumoniae* serotype K1 into K20. Virulence 2017;8(5): 487-493; https://doi.org/10.1080/21505594.2016.1228508

13. Shu HY, Fung CP, Liu YM, Wu KM, Chen YT, Li LH, Liu TT, Kirby R, Tsai SF. Genetic diversity of capsular polysaccharide biosynthesis in *Klebsiella pneumoniae* clinical isolates. Microbiology 2009; 155:4170-83; PMID:19744990; https://doi.org/10.1099/mic.0.029017-0

14. Lin JC, Chang FY, Fung CP, Xu JZ, Cheng HP, Wang JJ, Huang LY, Siu LK. High prevalence of phagocytic-resistant capsular serotypes of *Klebsiella pneumoniae* in liver abscess. Microbes Infect 2004; 6:1191-8; PMID:15487688; https://doi.org/10.1016/j.micinf.2004.06.003

15. Lin YT, Jeng YY, Chen TL, Fung CP. Bacteremic community-acquired pneumonia due to *Klebsiella pneumoniae*: clinical and microbiological characteristics in Taiwan, 2001-2008. BMC Infect Dis 2010; 10:307; PMID:20973971; https://doi.org/10.1186/1471-2334-10-307

16. Choi MJ, Ko KS. Loss of hyperviscosity and increased fitness cost in colistin-resistant *Klebsiella pneumoniae* sequence type 23 strains. Antimicrob Agents Chemother 2015; 59:6763-73; PMID:26282408; https://doi.org/10.1128/AAC.00952-15