Virulence 6:5, 407–409; July 2015; Published with license by Taylor & Francis Group, LLC

Klebsiella pneumoniae and the pyogenic liver abscess: implications and association of the presence of *rpmA* genes and expression of hypermucoviscosity

María Dolores Alcántar-Curiel[1,*] and Jorge A Girón[2]

[1]Facultad de Medicina; Universidad Nacional Autónoma de México; México DF, México; [2]Centro de Detección Biomolecular; Benemérita Universidad Autónoma de Puebla; Puebla, México

Keywords: ESBL, hypermucoviscosity, Klebsiella pneumoniae, pyogenic liver abscess, rmpA gene, virulence factors

Klebsiella pneumoniae is a common Gram-negative human opportunistic pathogen that causes both community and nosocomial infections primarily in immunocompromised hosts. This pathogen produces a wide range of infections including, pneumonia, bacteremia, urinary tract infection, meningitis, intra-abdominal infections, and community-acquired pyogenic liver abscess (PLA).[1-5]

Several virulence factors have been characterized clearly demonstrating their contribution to *K. pneumoniae* pathogenesis. The major virulence factors so far described are: a) the capsular polysaccharide (CPS), which confers the bacteria the ability to evade phagocytosis by immune cells and impedes bacterial killing by bactericidal complement serum;[6] b) the LPS O-antigen, prevents complement protein deposition and complement-associated serum lytic activity;[7] c) several pili or fimbrial adherence factors required for epithelial cell attachment and host colonization such as type 1 and type 3 pili, *K. pneumoniae* fimbriae 28 (KPF-28), the non-fimbrial adhesin CF29K, and the *E. coli* common pilus (ECP) have been described;[2,8] d) enterobactin and aerobactin siderophores, are required for a pathogen to establish infection when entering the hosts and enhance iron uptake by *K. pneumoniae*,[9] e) mucosity, a phenotype associated with a muco-polysaccharide network associated outside the capsule can facilitate mucosal colonization by *K. pneumoniae* and protects it from the interaction with anti-capsule-specific antibodies to evade phagocytosis.[10] Further, the hypermucoviscosity phenotype (HV) reported for certain strains are believed to increase the virulence of *K. pneumoniae*. The HV is regulated by the expression of 2 capsular polysaccharide genes: regulator of the mucoid phenotype gene (*rmpA*), a determinant controlling the CPS biosynthesis located in the chromosome or on the 180–220 Kb plasmid, and the plasmid-encoded transcriptional regulator of mucoid phenotype *rmpA2*. Iron-acquisition factors encoded on the same plasmid are also involved in regulation of the HV phenotype.[11-13] Studies in animal models have shown that the HV-phenotype of *K. pneumoniae* isolates is one of the major virulence factors required to produce bacteremia in diabetic mice and that other factors could be involved in the systemic spread of bacteria.[14] Another important point essential to the ability of *K. pneumoniae* to overcome antimicrobial therapies and to survive in demanding environments is multi-drug resistance, especially of isolates associated with nosocomial infections. One of the most common mechanisms of antimicrobial resistance of *K. pneumoniae* is the extended-spectrum β-lactamases (ESBL) production. ESBL-producing *K. pneumoniae* are rarely associated with community-acquired bacteremia and rarely express the HV phenotype.[15] However, a recent report in China showed that antimicrobial resistance of *K. pneumoniae* HV^+ variants has increased significantly.[12]

In 1986 a new invasive syndrome, primer pyogenic liver abscess (PLA), caused by *K. pneumoniae* was first described in Taiwan.[16] In the last 3 decades, this syndrome has been reported in patients from many Asian countries, South Africa, Australia, Europe, and America.[17,18] This community-acquired primary infection occurred primarily in healthy individuals and was associated with predisposing risk factors such as diabetes, malignancy, renal disease, and pneumonia.[19] PLA was caused by HV^+ variants of *K. pneumoniae*, and thus were designated as hypervirulent.[13] These isolates exhibit the potential to cause nosocomial infections including pneumonia, meningitis, endophtalmitis, and may also cause metastatic infections.[12]

Several controversies have risen regarding the ability of *K. pneumoniae* to express the HV phenotype and its association with the prevalence of the *rmpA/rmpA2* genes. Although some authors have

*Correspondence to: María Dolores Alcántar Curiel; Email: alcantar@unam.mx

Submitted: 03/05/2015; Revised: 03/05/2015; Accepted: 03/11/2015

http://dx.doi.org/10.1080/21505594.2015.1030101

Comment on: Yu et al. Low prevalence of rmpA and high tendency of rmpA mutation correspond to low virulence of extended spectrum β-lactamase-producing Klebsiella pneumoniae isolates. Virulence 2015; 6(2):162-72; http://dx.doi.org/10.1080/21505594.2015.1016703

reported that *rmpA* is present in the vast majority of HV^+ isolates, a small proportion of HV^+ isolates do not possess these genes.[20] Similarly, most of the HV-negative isolates do not carry *rmpA/rmpA2* but a small group of them may carry *rmpA* and/or *rmpA2*.[20,21] Another point that remains unclear is whether the HV phenotype correlates with virulence. For instance, while the majority of isolates exhibiting the HV phenotype are considered more virulent, some HV-negative isolates show greater ability to cause bacteremia in the diabetes mouse model.[15] Thus, it is apparent that virulence cannot be defined by the expression of HV alone. It is possible that HV is a key factor induced by specific host signals at different niches and could be important at certain stages during infection, for example during biofilm formation, particularly to evade the immune system and to colonize certain tissues within the host. This would explain, in part, the versatility of this pathogen to cause a myriad of human diseases.

In a recent issue of *Virulence*, Yu et al.[22] investigated why some *rmpA*-positive *K. pneumoniae* were HV-negative. To this aim, they performed nucleotide sequencing analysis of the *rmpA* and *rmpA2* genes in HV^- and HV^+ isolates. The comparative gene sequence analysis indicated the presence of frameshift mutations in *rmpA* or *rmpA2* genes in all of the HV^- isolates, but not in the HV^+ isolates. The authors suggested that mutations in *rmpA/rmpA2* influenced negatively, to some extent, the level of virulence associated with the HV phenotype of *K. pneumoniae* and also that these mutations are responsible for the poor association of HV with ESBL-producing *K. pneumoniae* isolates.

The emergence and spread of ESBL-producing *K. pneumoniae* strains raise important questions regarding optimal therapies for serious infections caused by these microorganisms.[23] *K. pneumoniae* causing nosocomial infections are ESBL-producing; however, hypervirulent *K. pneumoniae* variants causing PLA have been reported as non-ESBL-producing.[15] The possibility that *K. pneumoniae* isolates causing PLA can increase resistance to antimicrobials is a potential public health problem and a continuous threat. Recent studies indicate that hypervirulent isolates continue to be highly susceptible but have increased their degree of antimicrobial resistance over time.[12] Li et al. speculated why hypervirulent isolates exhibit low antimicrobial resistance, and suggested that these isolates perhaps cannot acquire resistance plasmids or may lose some resistance genes when they become hypervirulent.[12] Yu et al.[22] explained this controversy conducting conjugation experiments with ESBL-producing *K. pneumoniae* that exhibited the HV phenotype. They showed that the SHV-5 gene, one of the most prevalent ESBL genes and the *rmpA* gene are located on different plasmids. Based on their data they speculated that the ESBL-producing *K. pneumoniae* reduce genetic function of the *rmpA* system when the bacteria need to express ESBL genes during exposure to antibiotics. This is consistent with the general idea of bacterial metabolic economy to regulate gene expression in response to different host environment signals such as one under antibiotic selective pressure.

In sum, the epidemiological and molecular as well as experimental data from mouse models on infection indicate a strong correlation between the ability of PLA-causing *K.pneumoniae* strains to exhibit HV and to carry the *rmpA/rmpA2* genes. The studies discussed here highlight the need to deepen our understanding of the biological significance of HV as it relates to pathogenesis in the host. It is clear that further molecular and epidemiological characterization studies of potential factors that may contribute to virulence of *K. pneumoniae* causing PLA are needed. Chief to the clinical importance of the HV phenotype of *K. pneumoniae* strains worldwide is need to expand the number of ESBL- or non-ESBL-producing *K. pneumoniae* isolates originating from different geographic regions of the world and to determine the distribution of *rmpA* genes with or without genetic mutations in these strains. This opens an avenue for an interesting line of research on the genetics factors of the bacteria and/or the environmental cues that regulate *rmpA* gene function and the origin of its frameshift mutations. The investigation of the true role of the *rmpA* genes in expression of the HV phenotype and the virulence of PLA-causing strains calls for knock-out/complementation studies to fulfill molecular Koch's Postulates. Of vital importance is the need to stand alert and increase the surveillance of antimicrobial resistance of these isolates, particularly in different regions the world where the syndrome is prevalent for earlier detection and optimal treatment of patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

The authors acknowledge the support of Grant no. 104804-M from the Consejo Nacional de Ciencia y Tecnología México, for the research of virulence factors in *K. pneumoniae*.

References

1. Chang WN, Lu CH, Huang CR, Chuang YC, Tsai NW, Chang CC, Chen SF, Wang HC, Yang TM, Hsieh MJ, et al. Clinical characteristics of post-neurosurgical *Klebsiella pneumoniae* meningitis in adults and a clinical comparison to the spontaneous form in a Taiwanese population. Clin Neuro 2010;17:334-8; http://dx.doi.org/10.1016/j.jocn.2009.06.019.
2. Podschun R, Ullmann, U. *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11:589-603; PMID:9767057.
3. Ko WC, Paterson DL, Sagnimeni AJ, Hansen DS, Gottberg AV, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, et al. Community-acquired *Klebsiella pneumoniae* bacteremia: Global differences in clinical patterns. Emerg Infect Dis 2002;8:160-6; PMID:11897067; http://dx.doi.org/10.3201/eid0802.010025.
4. Tsai FC, Huang YT, Chang LY, Wang JT. Pyogenic liver abscess as endemic disease, Taiwan. Emerg Infect Dis 2008;14:1592-600; PMID:18826824; http://dx.doi.org/10.3201/eid1410.071254.
5. Hsieh PF, Wu MC, Yang FL, Chen CT, Lou TC, Chen YY, Wu SH, Sheu JC, Wang JT. D-galactan II is an immunodominant antigen in O1 lipopolysaccharide and affects virulence in *Klebsiella pneumoniae*: implication in vaccine design. Front Microbiol 2014; 5:608; PMID:25477867; http://dx.doi.org/10.3389/fmicb.2014.00608.
6. Cortés G, Borrell N, de Astorza B, Gómez C, Sauleda J, Albertí S. Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of *Klebsiella pneumoniae* in a murine model of pneumonia. Infect Immun 2002;70:2583-90; http://dx.doi.org/10.1128/IAI.70.5.2583-2590.2002.
7. Tichaczek-Goska D, Witkowska D, Cisowska A, Jankowski S, Hendrich AB. The bactericidal activity of normal human serum against *Enterobacteriaceae* rods with lipopolysaccharides possessing O-antigens composed of mannan. Adv Clin Exp Med 2012;21:289-299; PMID:23214191.
8. Alcántar-Curiel MD, Blackburn D, Saldaña Z, Gayosso-Vázquez C, Iovine N, De la Cruz MA, Girón

JA. Multi-functional analysis of *Klebsiella pneumoniae* fimbrial types in adherence and biofilm formation. Virulence 2013;4:129-138; http://dx.doi.org/10.4161/viru.22974.
9. Siu LK, Yeh KM, Lin JC, Fung CP, Chang FY. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. Lanced Infect Dis 2012;12:881-87; http://dx.doi.org/10.1016/S1473-3099(12)70205-0.
10. Nassif X, Fournier JM, Arondel J, Sansonetti PJ. Mucoid phenotype of *Klebsiella pneumoniae* is a plasmid-encoded virulence factor. Infect Immun 1989;57:546-552; PMID:2643575.
11. Chen YT, Chang HY, Lai YC, Pan CC, Tsai SF, Peng HL. Sequencing and analysis of the large virulence plasmid pLVPK of *Klebsiella pneumoniae* CG43. Gene 2004; 337;189-198; PMID:15276215; http://dx.doi.org/10.1016/j.gene.2004.05.008.
12. Li W, Sun G, Yu Y, Li N, Chen M, Jin R, Jiao Y, Wu H. Increasing occurrence of antimicrobial-resistant hypervirulent (Hypermucoviscous) *Klebsiella pneumoniae* isolates in China. Clin Infect Dis 2014;58:225-32; PMID:24099919; http://dx.doi.org/10.1093/cid/cit675.
13. Yu WL, Ko WC, Cheng KC, Lee CC, Lai CC, Chuang YC. Comparison of prevalence of virulence factors for *Klebsiella pneumoniae* liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. Diagn Microbiol Infect Dis 2008;62:1-6; PMID:18486404; http://dx.doi.org/10.1016/j.diagmicrobio.2008.04.007.
14. Lin YC, Lu MC, Tang HL, Liu HC, Chen CH, Liu KS, Lin C, Chiou CS, Chiang MK, Chen CM, et al. Assessment of hypermucoviscosity as a virulence factor for experimental *Klebsiella pneumoniae* infections: comparative virulence analysis with hypermucoviscosity-negative strain. BMC Microbiol 2011;11:50; PMID:21385400; http://dx.doi.org/10.1186/1471-2180-11-50.
15. Lin TL, Tang SI, Fang CT, Hsueh PR, Chang SC, Wang JT. Extended-spectrum β-lactamase genes of *Klebsiella pneumoniae* strains in Taiwan: Recharacterization of *shv-27, shv-41*, and *tem-116*. Microb Drug Resist 2006;12:12-15.21; PMID:16584302; http://dx.doi.org/10.1089/mdr.2006.12.12.
16. Liu YC, Cheng DL, Lin CL. *Klebsiella pneumoniae* liver abscess associated with septic endophthalmitis. Arch Intern Med 1986; 146: 1913-1916; PMID:3532983; http://dx.doi.org/10.1001/archinte.1986.00360220057011.
17. Chuang YP, Fang CT, Lai SY, Chang SC, Wang JT. Genetic determinants of capsular serotype K1 of *Klebsiella pneumoniae* causing primary pyogenic liver abscess. J Infect Dis 2006; 193:645-54; PMID:16453259; http://dx.doi.org/10.1086/499968.
18. Vila A, Cassata A, Pagella H, Amadio C, Yeh KM, Chang FY, Siu LK. Appearance of *Klebsiella pneumoniae* liver abscess syndrome in Argentina: Case report and review of molecular mechanisms of pathogenesis. Open Microbiol J 2011;5:107-113; PMID:22145012; http://dx.doi.org/10.2174/1874285801105010107.
19. Tsai FC, Huang YT, Chang LY, Wang JT. Pyogenic liver abscess as endemic disease, Taiwan. Emerg Infect Dis 2008;14:1592-1600; PMID:18826824; http://dx.doi.org/10.3201/eid1410.071254.
20. Lee CH, Liu JW, Su LH, Chien CC, Li CC, Yang KD. Hypermucoviscosity associated with *Klebsiella pneumoniae*-mediated invasive syndrome: a prospective cross-sectional study in Taiwan. Int J Infect Dis 2010;14:e688-e92; PMID:20547084; http://dx.doi.org/10.1016/j.ijid.2010.01.007.
21. Yu WL, Ko WC, Cheng KC, Lee HC, Ke DS, Lee CC, Fung CP, Chuang YC. Association between *rmpA* and *magA* genes and clinical syndromes caused by *Klebsiella pneumoniae* in Taiwan. Clin Infect Dis 2006;42:1351-8; PMID:16619144; http://dx.doi.org/10.1086/503420.
22. Yu WL, Lee MF, Tang HJ, Chang MC, Chuang YC. Low prevalence of *rmpA* and high tendency of *rmpA* mutation correspond to low virulence of extended spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates. Virulence 2015;6:162-72; PMID:25830726.
23. Alcántar-Curiel D, Tinoco JC, Gayosso C, Carlos A, Daza C, Perez-Prado MC, Salcido L, Santos JI, Alpuche-Aranda CM. Nosocomial bacteremia and urinary tract infections caused by extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* with plasmids carrying both SHV-5 and TLA-1 genes. Clin Infect Dis 2004;38:1067-1074; http://dx.doi.org/10.1086/382354.