Case Report

**Klebsiella pneumoniae** K2 producer of pyogenic liver abscess associated with biliary communication

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**Abstract**

Introduction: Hypervirulent strains of *Klebsiella pneumoniae* have gained clinical and epidemiological interest because of their capacity to cause severe and life-threatening infections.

Methodology: We report a case involving infection with a hypervirulent *K. pneumoniae* K2 strain that caused liver abscess in a young woman with type 1 diabetes in Mexico.

Results: The infection was found to be associated with biliary tract communication. The virulence factors and capsular serotypes were identified by polymerase chain reaction analysis. After guided drainage and directed antibiotic treatment, the infection resolved and the patient recovered.

Colonization of the gastrointestinal tract by hypervirulent *K. pneumoniae* strains, together with the presence of comorbidity, such as diabetes are important factors that contribute to the development of liver abscess.

Conclusions: The identification of virulent clones is important to understand the pathogenicity and improve control of infections in the patients.

**Key words:** infection; colonization; Klebsiella; abscess; virulence; K2.

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**Introduction**

The worldwide human nosocomial pathogen *Klebsiella pneumoniae* is a Gram-negative bacillus belonging to the Enterobacteriaceae family. This bacterium is one of the principal pathogens causing nosocomial infections and are responsible for respiratory, urinary, and gastrointestinal tract infections [1]. *K. pneumoniae* liver abscess (KLA) is an emerging infection worldwide that can cause *K. pneumoniae* invasive syndrome characterized by liver abscesses, bacteremia, and metastatic infections of the eyes and central nervous system [2].

Pyogenic liver abscesses in people with diabetes without a biliary tract disorder can be caused by the hypervirulent *K. pneumoniae* (hvKpn) pathotype and was first described in Asia in the 1980s. Lin *et al.* [3] suggested colonization of the gastrointestinal tract by *K. pneumoniae* via the portal vein as the primary cause of liver abscess after isolating this bacterium in feces from a healthy Chinese person. The major risk factors for the development of KLA caused by hvKpn are diabetes, cholelithiasis, and the dietary composition. Diabetic patients with poor glycemic control may have impaired neutrophil phagocytosis. KLA infection in people with diabetes, especially if poorly controlled, increases the risk of hepatic vein thrombophlebitis and gas production caused by changes in the local microenvironment that favor bacterial growth [4,5].

Cases of KLA caused by hvKpn have also been reported from North America and Europe, but there are few reports from Latin America. The hvKpn has been described with serotypes K1, K5, and K19 in Brazil, K1 in Argentina, and K2 in Mexico [6–8]. The reason for higher prevalence in Asia is unclear, but it may be related to the ethnicity and differences in the virulence of the bacterium itself.

The hvKpn strains present more frequently as the capsular serotypes K1 and K2, which are associated with the phenotype of hypermucoviscosity (hmv). The hvKpn phenotype is mediated by the presence of virulence genes such as the regulators of the mucoid phenotype (*rmpA* and *rmpA2*) and of siderophore biosynthesis such as *iroB* (salmochelin), *entB* (enterobactin), *ybtQ* (yersiniabactin), and *iucA*.
(aerobactin); the latter is considered to be the most important [9]. A putative transporter (peg-344) is considered to be a biomarker for the hvKpn strain, and it has been suggested that this gene can be used to differentiate between hypervirulent and typical K. pneumoniae [10].

The hyperproduction of capsule in hvKpn strains increases its resistance to humoral defenses, killing by complement, and phagocytosis by macrophages and neutrophils. Thus, hvKp has a potential invasive phenotype and could produce liver abscesses [11]. Most hvKpn isolates with serotype K1 from clonal group (CG) 23 have been described in Taiwan, Singapore, and China. CG23 includes the sequence type (ST) 23, ST26, ST57, and ST1633. This CG was originally associated with the lack of acquired antimicrobial resistance, although this has changed recently, and the resistance to cephalosporins and carbapenems is increasing [12].

Case presentation

A 30-year-old woman was admitted to the intensive care unit of Medica Sur Hospital, Mexico City, Mexico, in September 2019. The patient’s comorbidities included type 1 diabetes without intra-abdominal or biliary tract infection. She was previously hospitalized for diabetic ketoacidosis and by the time of this hospital admission she was on insulin glargine (20 units per day) and insulin lispro (15 units’ pre-meal). The patient reported consumption of Ganoderma lucidum mushroom powder in tea several times during the month before the onset of her symptoms. There was no history of travel to Asia.

On admission, the patient was alert but exhibited fatigue, generalized jaundice, nausea, and oral intolerance. On physical exam, her vital signs were blood pressure 80/50 mmHg, heart rate 115 beats/min, and body temperature 39.3 °C. She had abdominal pain in the right upper quadrant. Laboratory data showed white blood cell count $41 \times 10^3/\mu L$ (neutrophils $36.9 \times 10^3/\mu L$, lymphocytes $1.6 \times 10^3/\mu L$), hemoglobin level 8.3 g/dL, platelet count $327 \times 10^3/\mu L$, C-reactive protein level 162 mg/L, creatinine level 1.55 mg/dL, and HbA1c (glycated hemoglobin) level 10.1%. Serum aspartate aminotransferase and alanine aminotransferase levels were 287 U/L and 33 U/L, respectively. Serum direct and indirect bilirubin were 11.39 mg/dL and 12.22 mg/dL, respectively. A bedside hepatic ultrasound revealed three heterogenic images

Figure 1. Computed tomography images showing multiple liver abscesses before drainage (a–c, dotted circle). After drainage, the abscesses have resolved (d, arrow).
related to hepatic abscesses, the biggest was localized on segment VI with 70 mm × 57 mm × 55 mm; the remaining two abscesses were localized on segments VII with 55 mm × 31 mm × 17 mm, and 47 mm × 40 mm × 39 mm respectively (Figure 1). The patient underwent surgical drainage of the largest abscess where approximately 300 ml of purulent content was reported. An initial antimicrobial scheme was based on intravenous ceftriaxone plus metronidazole.

Purulent samples were cultured and pan-susceptible *K. pneumoniae* was isolated. The antibiotic therapy was adjusted to ertapenem. The *Entamoeba* IgG antibody test was negative (1.37 U, cutoff value 0–8.99 U), and the stool culture was also negative.

Days later a computed tomography (CT) scan of the abdomen revealed images compatible with the presence of residual liver abscesses; the most representative were two abscesses in segment VII measuring 45 × 43 mm and 58 × 43 mm and another across segment VI that was in close contact with the ipsilateral kidney (Figure 1). Liver cysts and cholelithiasis were not seen in the abdominal CT images. Those latter abscesses were drainage by percutaneous aspiration and placement of a catheter with the same microbiology results as the samples.

Several days later the clinical and laboratory parameters improved, a new CT scan of the abdomen revealed reduction of more than 90% of the abscesses. The patient was discharged with a prescription for ertapenem for 14 more days as an outpatient antimicrobial therapy.

**Bacterial isolates and antimicrobial susceptibility**

Drainage samples were collected aseptically from the patient with liver abscesses. The bacteria isolated from the abscesses were cultivated and subjected to MALDI-TOF-MS (Becton-Dickinson, Heidelberg, Germany) analysis, and identified as *K. pneumoniae*. Antimicrobial susceptibility was identified using a BD Phoenix™ automated system (Becton-Dickinson, Maryland, USA), and the results were interpreted using CLSI M100 breakpoints [14].

**Determination of the hvKpn phenotype**

The *K. pneumoniae* 3322874 isolate was plated in blood agar using a bacteriology loop and grown in a MacConkey plate for 18 h at 37°C. The formation of a viscous string > 10 mm was defined as string test positive [13]. The phenotype of the isolate spread by the bacteriology loop did not necessarily equate to the hvKpn phenotype [8].

**Detection of virulence factors**

The peg-344, iroB, iucA, entB, rmpA, and rmpA2 virulence factors and capsular serotypes (K1, K2, K5, K20, K54, and K57) were identified by polymerase chain reaction (PCR), and the primer sets are described in Table 1.

Table 1. Primer sequences of the virulence determinants.

| Gene and serotype | Sequence (5′ – 3′) | Reference |
|-------------------|--------------------|-----------|
| rmpA              | rmpA-F: ACTGGGCTACCTCTGCTTCA rmpA-R: CTTGCATGAGCCATCTTTCA | [15] |
| rmpA2             | rmpA2-F: CTCTATGTGCAATAAGGATGTT rmpA2-R: CCTCCTGGAGAAGACATT | [15] |
| entB              | entB-F: GATGAAGACGATACCGTGC entB-R: ACCGAATCCAGACCGTAGTC | [16] |
| iroB              | iroB-F: ATCTCATTCATCTACCCCGGTC iroB-R: GGTTGCGCGTCGTTTCAA | [16] |
| iucA              | iucA-F: AATCAATGGCTATTCGCCGCTG iucA-R: CGCTTCACCTCTTTTCACTGACAG | [16] |
| peg-344           | F1: CTTGAAAACATCTCCCTGACAGTC R1: CCAGC GAAGAAATACCCAGTGCG | [17] |
| wzy_K1            | wzyK1-F: GGTTGCTCTTTTACATCATTGC wzyK1-R: GCAATGGCCTTTGCCTTTAG | [18] |
| wzy_K2            | wzyK2-F: GACCCGATATTCACTGACAGAG wzyK2-R: CACTGAAAGGATCGTTAGCAGG | [19] |
| wzy_K5            | wzyK5-F: TGGTAGTGATGCTCGCCGA wzyK5-R: CCGTGAACCCACCCTTGAG | [19] |
| wzy_K20           | wzyK20-F: GCGGAGACCTTTGGAAAAAGC wzyK20-R: TCATTTACACCTGTTCC | [18] |
| wzy_K54           | wzyK54-F: TTACCTCAGAGCGTGGTAGAG | [18] |
| wzy_K57           | wzyK57-F: CTACCGCTGAAGGATGTCACT wzyK57-R: CACTAACCCAGAAAGTCGAG | [15] |
**Pathogenicity assay**

The pathogenicity of the isolate was evaluated using an in vivo experiment. The identification of the median lethal dose (LD₅₀) was determined in an experiment involving five groups of six female BALB/c mice. Mice were injected intraperitoneally with 100 µL of bacteria in the range of 100–100,000 CFU/mL [22] and monitored for 15 days.

**Outcomes**

CT-guided needle puncture with aspirated drainage is currently the first-line treatment of liver abscesses. The CT images for this patient are shown in Figure 1. Needle aspiration also allows the collection of a purulent sample for analysis. The *K. pneumoniae* isolate was susceptible to all antibiotics tested except for ampicillin (MIC > 16 µg/mL), which indicates intrinsic resistance. The mucoviscosity phenotype was observed in the agar plate colonies, but the string test was negative. The isolate was positive for the presence of K2, *rmpA*, *iroB*, *iucA*, *entB*, and *peg-344* hypervirulence factors (Table 1). *K. pneumoniae* 3322874 corresponds to ST380. The pathogenicity experiment using the isolate showed an LD₅₀ of 100 CFU/mL, and mice showed signs of illness or died 48 h after inoculation (Table 2).

**Discussion**

*K. pneumoniae* liver abscesses are more common in men and adults between the ages of 60 and 70 years. The risk of abscess increases when there are comorbidities such as diabetes mellitus or biliary tract or gastrointestinal disease [23]. Underlying conditions like diabetes mellitus or impaired fasting glucose is considered the major risk factor for liver abscesses in different Taiwan series [24,25,26]. It has been suggested that potential presence of pyogenic liver abscess should be checked in the case of patients with fever of unknown origin, where *K. pneumoniae* has been identified as the main pathogen, and the patient has comorbidities such as diabetes or biliary disease [27,28].

The presence of hvKpn can lead to complications that may have a poor prognosis, such as meningitis, endophthalmitis, fat liver, or cancer [29]. Moreover, the hvKpn K2 serotype is strongly associated with resistance to lysis caused by complement [30]. Most organisms isolated from KLA have low resistance to antibiotics, as observed in this case. Therefore, the rapid detection of antibiotic susceptibility and hypervirulent phenotype is needed to be able to choose the appropriate treatment and limit the risk of adverse consequences. Antibiotic treatment, including percutaneous drainage, has been shown to be highly effective [31] and to lead to better outcomes. Our patient required percutaneous drainage; the pigtail catheter was left in place and the patient was treated with parenteral and oral antibiotics. MLST analysis has shown that hvKpn K2 belongs to the potentially invasive ST380 [32,33]. ST380 is emerging as a cause of severe community-acquired infections associated with diabetes mellitus and Asian origin such as main risk factors. The hvKpn ST380 has been isolated from urine, surgical wounds, sputum, and blood in patients aged > 60 years and is associated with liver hepatic abscesses [34].

The patient in our study was a young woman with diabetes who developed KLA with type 1 diabetes and no previous abdominal surgery. Mortality rates up to 42% have been reported for KLA, and survivors may experience serious sequelae [35] despite the low level of antibiotic resistance of *K. pneumoniae*. These findings were consistent with the data obtained in the mouse pathogenicity assays, where the LD₅₀ at 48 h after infection was 100 CFU/mL.

It is important to study bacteria that show a hypervirulent phenotype because they increase the risk of developing short-term complications such as meningitis or pneumonia or long-term complications such as colorectal cancer [2,29]. Fortunately, these complications can be limited or prevented because at present, these phenotypes are susceptible to antibiotics.

| Table 2. Phenotypic and genotype characteristic of hypervirulent *K. pneumoniae* clinical isolate |
|----------------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Strain                               | Origin of sample                | String test     | Capsular genotype | *rmpA*/*rmpA2* | *iucA*/*iroB*/*entB* | *peg-344* | MLST              | LD₅₀ (CFU/mL) | Phenotype        |
|----------------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 3322874                               | Liver abscess                   | K2              | +/-             | +/-             | +               | ST380          | 100             | Hypervirulent    |

MLST: MultiLocus Sequence Typing; CFU: colony-forming unit.
Despite the fact that there is no evidence that *K. pneumoniae* is a digestive tract commensal, we consider that the presence of hypervirulent strains coupled with the uncontrolled diabetes are factors that lead to the production of liver abscesses in young patients. In addition, we considered that the patient’s consumption of *G. lucidum* may have been responsible for the hepatotoxicity, because the liver chemistry can mimic acute cholangitis after consumption of *G. lucidum* [36]. However, no cases of infections related to the consumption of this dry preparation of fungus have been reported. Finally, studying the phenotypic and genotypic characteristics of bacteria can be useful for identifying other pathologies, such as the association between *K. pneumoniae* strains and fatty liver disease, which involves successive complications such as inflammation, fibrosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma [37,38].

**Conclusions**

Thus, hypervirulent *K. pneumoniae* is a highly virulent strain related to hepatic abscess in particular in diabetic patients. In Mexico, hvKpn *K. pneumoniae* is susceptible to antibiotics. However, with the increase in cases of drug-resistant Enterobacteriaceae, the evolution of the susceptibility pattern should be monitored to prevent hypervirulent *K. pneumoniae* from becoming an epidemiological threat.

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**References**

1. Paczosa MK, Mecsas J (2016) *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiol Mol Biol Rev 80: 629–661.
2. Fung CP, Chang FY, Lee SC, Hu BS, Kuo BL, Liu CY, Ho M, Siu LKA (2002) A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis? Gut 50: 420–424.
3. Lin YT, Siu LK, Lin JC, Chen TL, Tseng CP, Yeh KM, Chang FY, Fung CP (2012) Seroepidemiology of *Klebsiella pneumoniae* colonizing the intestinal tract of healthy Chinese and overseas Chinese adults in Asian countries. BMC Microbiol 12: 13.
4. Liu C, Guo J (2019) Hypervirulent *Klebsiella pneumoniae* (hypermucoviscous and aerobactin positive) infection over 6 years in the elderly in China: antimicrobial resistance patterns, molecular epidemiology and risk factor. Ann Clin Microbiol Antimicrob 18: 4.
5. Jun JB (2018) *Klebsiella pneumoniae* liver abscess. Infect Chemother 5: 210–218.
6. Moura Q, Esposito F, Fernandes MR, Espinoza-Muñoz M, Souza TA, Santos SR, Cerdeira L, Cassettari V, Lincopan N (2017) Genome sequence analysis of a hypermucoviscous/hypervirulent and MDR CTX-M-15/K19/ST29 *Klebsiella pneumoniae* isolated from human infection. Pathog Dis 75: 121.
7. Vila A, Cassata A, Pagella H, Amadio C, Yeh KM, Chang FY, Siu LK (2011) Appearance of *Klebsiella pneumoniae* liver abscess syndrome in Argentina: case report and review of molecular mechanisms of pathogenesis. Open Microbiol J 5: 107–113.
8. Catalán-Nájera JC, Barrios-Camacho H, Duran-Bedolla J, Sagal-Prado A, Hernández-Castro R, García-Méndez J, Morfin-OteroMorfin-Otero R, Velázquez-Larios MDR, Ortiz-Navarrete V, Gutierrez-Xicotencatl L, Alpuche-Aranda C, Silva-Sánchez J, Garza-Ramos U (2020) Molecular characterization and pathogenicity determination of hypervirulent *Klebsiella pneumoniae* clinical isolates serotype K2 in Mexico”. Diagn Microbiol Infect Dis 94: 316–319.
9. Russo TA, Olson R, MacDonald U, Beaman J, Davidson BA (2015) Aerobactin, but not yersiniabactin, salmochelin, or enterobactin, enables the growth/survival of hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* ex vivo and in vivo. Infect Immun 83: 3325–3333.
10. Russo TA, Olson R, Fang CT, Stoesser N, Miller M, MacDonald U, Hutson A, Barker JH, La Hoz RM, Johnson JR I (2018) Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical *K. pneumoniae*. J Clin Microbiol 56: e00776-18.
11. Lin JC, Chang FY, Fung CP, Xu JZ, Cheng HP, Wang JJ, Huang LY, Siu LK (2004) High prevalence of phagocytic-resistant capsular serotypes of *Klebsiella pneumoniae* in liver abscess. Microbes Infect 6: 1191–1198.
12. Lam MMC, Wyres KL, Duchêne S, Wick RR, Judd LM, Gan YH, Hoh C-H, Archuleta S, Molton JS, Kalimuddin S, Koh T-T, H, Passet V, Brisse S, Holt KE (2018) Population genomics of hypervirulent *Klebsiella pneumoniae* clonal-group 23 reveals early emergence and rapid global dissemination. Nat Commun 9: 2703.
13. Lee HC, Chuang YC, Yu WL, Lee NY, Chang CM, Ko NY, Wang LR, Ko WC (2006) Clinical implications of hypermucoviscosity phenotype in *Klebsiella pneumoniae* isolates: association with invasive syndrome in patients with community-acquired bacteremia. J Intern Med 259: 606–614.
14. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.
15. Lee CH, Liu JW, Su LH, Chien CC, Li CC, Yang KD (2010) Hypermucoviscosity associated with *Klebsiella pneumoniae*-mediated invasive syndrome: a prospective cross-sectional study in Taiwan. Int J Infect Dis 14: e688–e692.
16. Russo TA, Olson R, MacDonald U, Metzger D, Maltese LM, Drake EJ, Gulick AM (2014) Aerobactin mediates virulence and accounts for increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*. Infect Immun 82: 2356–2367.
17. 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: e00776-18.
18. Pan YJ, Lin TL, Chen YH, Hsu CR, Hsieh PF, Wu MC, Wang JT (2013) Capsular types of *Klebsiella pneumoniae* revisited by wzc sequencing. PLoS One 8: e80670.
19. Turton JF, Perry C, Elgohari S, Hampton CV (2010) PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. J Med Microbiol 59: 541–547.

20. 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.

21. Institut Pasteur (2021) Klebsiella sequence typing. Available: https://bigsdb.pasteur.fr/klebsiella/klebsiella.html. Accessed: 2 June 2021.

22. Reed LJ, Muench H (1938) A simple method of estimating fifty per cent endpoint. Am J Epidemiol 27: 493–497.

23. Wang WJ, Tao Z, Wu HL (2018) Etiology and clinical manifestations of bacterial liver abscess: a study of 102 cases. Medicine (Baltimore) 97: e12326.

24. Zhang ZD, Liang YJ, Yin DL, Lee L, Jiang HC, Liu LX (2012) Liver abscesses in adult patients with and without diabetes mellitus: an analysis of the clinical characteristics, features of the causative pathogens, outcomes and predictors of fatality: a report based on a large population, retrospective study in China. Clin Microbiol Infect 18: E314–330.

25. Liu Y, Wang YJ, Jiang W (2013) An increasing prominent disease of Klebsiella pneumoniae liver abscess: etiology, diagnosis, treatment. Gastroenterol Res Pract: 258514.

26. Huang WK, Chang JW, See LC, Tu HT, Chen JS, Liaw CC, Lin YC, Yang TS (2012) Higher rate of colorectal cancer among patients with pyogenic liver abscesses with Klebsiella pneumoniae than those without: an 11-year follow-up study. Colorectal Dis 14: e794–801.

27. Liu K, Yeh K, Lin J, Fung C, Chang F (2012) Klebsiella pneumoniae liver abscess: a new invasive syndrome. Lancet Infect Dis 12: 881–887.

28. Heneghan HM, Healy NA, Martin ST, Ryan RS, Nolan N, Traynor O, Waldron R (2011) Modern management of pyogenic hepatic abscess: a case series and review of the literature. BMC Res Notes 4: 80.

29. Hentzen M, Rosman J, Decré D, Brenkle K, Mendes-Martins L, Mateu P (2017) Seven hypervirulent ST380 Klebsiella pneumoniae septic localizations. Med Mal Infect 47: 171–173.

30. Shon AS, Bajwa RPS, Russo T (2013) Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: a new and dangerous breed. Virulence 4: 107–118.

31. Syn WK, Teibbery V, Choi SS, Diehl AM (2009) Similarities and differences in the pathogenesis of alcoholic and nonalcoholic steatohepatitis. Semin Liver Dis 29: 200–210.

32. De Medeiros IC, de Lima JG (2015) Is non-alcoholic fatty liver disease an endogenous alcoholic fatty liver disease? A mechanistic hypothesis. Med Hypotheses; 5: 148–152.