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Case report

Pyogenic liver abscess caused by an atypical hypervirulent *Klebsiella pneumoniae* K1-ST23 in Mexico

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ABSTRACT

Hypervirulent *K. pneumoniae* infection has been raising worldwide and is one of the major causes of community-acquired pyogenic liver abscess. We described a case report of pyogenic liver abscess caused by an atypical hypervirulent (non-hypermucoviscous) *K. pneumoniae* K1 ST23 in a diabetic Asian patient who resided in Mexico. The susceptibility to antimicrobials, pathogenicity, molecular and genomic analysis were determined. A man from Guangdong (China) with a recent diagnosis of diabetes mellitus was admitted to the hospital, and he denied traveling in the last 3 months. A computed tomography revealed a right lobe liver abscess. On the third day after admission a *Klebsiella pneumoniae* isolate (14652) was obtained. The isolate corresponded to a susceptible *K. pneumoniae* with capsular type K1 and ST23 (CG23) and exhibited a non-hypermucoviscous phenotype. The isolate 14652 was genetically related to the globally distributed lineage ST23-KL1. This study describes the first case in Mexico of *K. pneumoniae* capsular type K1 and ST23 with an atypical hypervirulent phenotype.

Introduction

Hypervirulent *K. pneumoniae* (hvKp) causes severe life-threatening diseases such as endophthalmitis, meningitis, brain, and liver abscess in both immunocompromised and healthy patients [1,2]. Its identification by clinicians is critical for treatment and patient management. The following criteria have been proposed for its definition: i) the presence of the *rmpA* and *iucA* genes: the first increases the production of capsule and the second encodes for the dominant siderophore in hvKp, ii) the presence of organ lesions suggestive of invasive infections, and iii) the presence of the hypermucoviscous phenotype which is determined by a positive "string test" [1,3]. However, these parameters as a whole are not always taken into account when identifying hvKp, and for its simplicity the string test has been adopted in many laboratories as the sole means for identifying hvKp. Several studies have documented that this assay is not accurate and may lead to false negatives (non-hypermucoviscous but hypervirulent Kp), especially in low-prevalence regions [1,4].

Rodriguez-Medina and colleagues characterized isolates of *K. pneumoniae* collected from Mexican health care settings in which they reported non-hypermucoviscous hvKp isolates belonging to the ST23-K1, the dominant hypervirulent (hv) lineage, the ST380-K2 and the ST3999-K2 that were termed atypical hypervirulent due to the low virulence in animal models and low production of capsule and hypermucoviscosity [5]. These isolates were considered hypervirulent because of the genomic traits and phylogenetic position.

Here, we describe the first case report of a pyogenic liver abscess caused by an atypical hypervirulent *K. pneumoniae* capsular type K1, its microbiological and virulence traits and the genomic analysis confirming its the genetic relationship to the globally distributed lineage ST23-

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K1.

Case

A 45-year-old man from Guangdong (China), with a recent diagnosis of diabetes mellitus was admitted to the hospital presenting 6/10 right upper quadrant abdominal pain, nausea, vomiting, fever, and chills for the past 7 days. He had not received any antimicrobial agents or been hospitalized in the previous 90 days. He has been living in Mexico City since 2017, but he denies having traveled in the last 3 months. He works as a merchant of electronic products.

When admitted, he was febrile and his vital signs indicated sepsis; his body temperature was 38.5 °C, heart rate 110 beats per minute, blood pressure 110/60 mmHg, respiratory rate 17 breaths/minute, and peripheral oxygen saturation 95 % (SpO_2).

An abdominal examination revealed hepatomegaly with pain caused by inspiration and liver percussion. Although his abdomen was soft with no tenderness, the spleen was not palpable.

Peripheral blood analysis yielded the following results: white blood cell count, 18.2 × 10^9/L (neutrophils 94.7 %); hemoglobin level, 12.7 mg/dL; and platelet count, 439 10^9/dL. C-reactive protein levels were 19.7 mg/L and procalcitonin (PCT) levels were 50.28 ng/ml. His serum aspartate aminotransferase and alanine aminotransferase levels were normal, and his glycosylated hemoglobin A1 (HbA1C) level was 13.9 %. A computed tomography (CT) scan with contrast of the chest and abdomen was ordered revealing a right lobe liver abscess in segments VI, VII and VIII, of 13.48 × 11.22 × 11.08 cm in size (Fig. 1).

He was admitted to the general medicine floor, empirical treatment was given immediately with ceftriaxone (1 g every 12 h), metronidazole (500 mg every 8 h), fluid resuscitation consisting of crystalloids, and blood and stool cultures were ordered. Three sets of blood cultures showed no bacterial growth. Stool examination was also negative for amoebae, ova, or cysts. Bacterial culture to check for the presence of bacterial pathogens in the stool was not performed.

Ultrasound-guided puncture was performed 6 h after admission, yielding 800 ml of thick pus. Fluid was collected and pathogen culture was obtained.

Five days after admission he became tachypneic, tachycardic and hypoxemic with oxygen saturations (SpO_2) was in the 86 %s. On examination breath sounds were reduced in right lower lobe, the patients received oxygen supplementation through simple face mask with flow rate of 8–10 l/min. Chest radiography and CT of thorax and abdomen showed right loculated pleural effusion and some associated atelectasis, with a residual complex hepatic mass in segment VII (Fig. 2).

Thoracocentesis with a chest tube drainage was performed by thoracic surgery team. About 1400 ml of a yellowish opaque liquid was evacuated from the pleural cavity. Pleural fluid analysis confirmed an exudative effusion, microbiological analysis of the pleural fluid resulted negative. Patient remained with the pleural chest tube for 7 days, with complete recovery in oxygen levels.

As there was considerable clinical and biochemical improvement, he was discharged to complete a 3-week further course of orally administered cefixime and metronidazole, he did not require further aspirations. The patient received a total of 6 weeks antibiotic treatment with intravenous ceftriaxone (2 g every 24 h) and oral metronidazole (500 mg every 8 h) with a notable improvement in laboratory and image studies, a repeat tomography performed at completion of antibiotic therapy revealed resolving abscess in segment VII measuring 2.5 × 3.4 cm in size containing less of 10 cc (Fig. 3).

Bacterial isolates and antimicrobial susceptibility

Drainage samples were collected aseptically from the patient with liver abscesses. Gram-negative rods were observed on Gram stain, and on the third day after admission Klebsiella pneumoniae 14652 isolate was confirmed by molecular test. Briefly, initial denaturation step at 94 °C for 5 min, 30 cycles consisting of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 50 s and final extension step at 72 °C for 2 min. Primers concentrations of 25 pmol were used for the Kv275 and Kq372 targets and 50 pmol for the Kp650 for the multiplex reaction in a 50-ul final volume [6]. The strain was determined to be resistant to ampicillin and tetracycline, but sensitive to piperacillin/tazobactam, aztreonam, cefotaxime, ceftazidime, imipenem; according to CLSI M100 (32nd Edition) breakpoints [7].

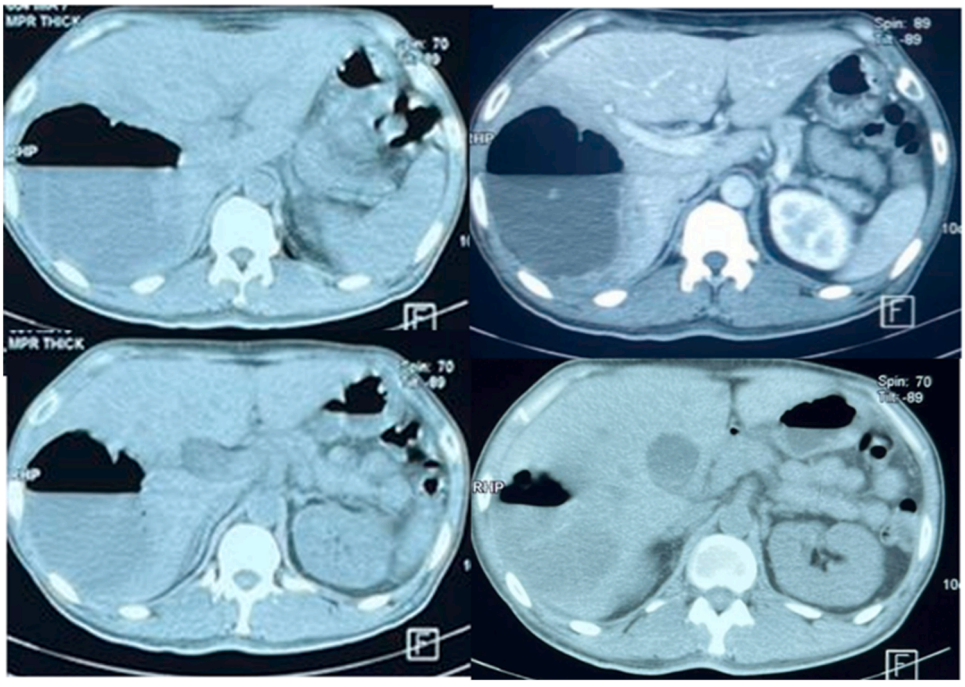

Fig. 1. Abdominal computed tomography (CT) scan with contrast revealing a right lobe liver abscess in segments VI, VII and VIII, of 13.48 × 11.22 × 11.08 cm in size.

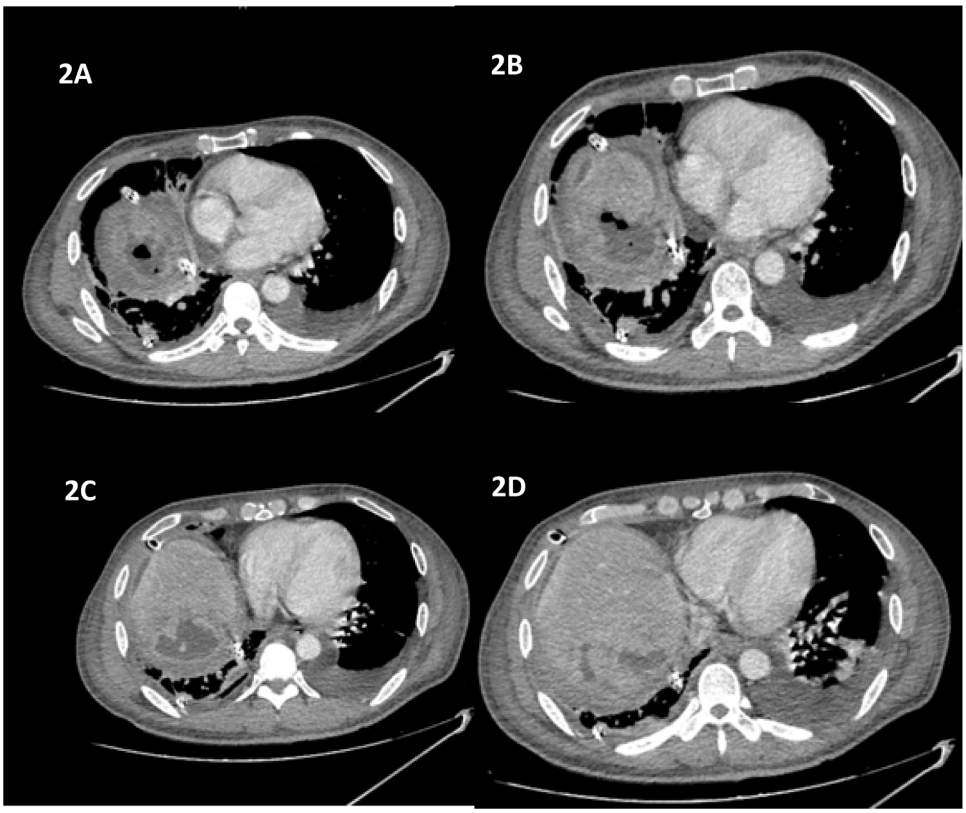

Fig. 2. Contrasted thoracic and abdominal tomography with right loculated pleural effusion and residual complex hepatic mass in segment VII.

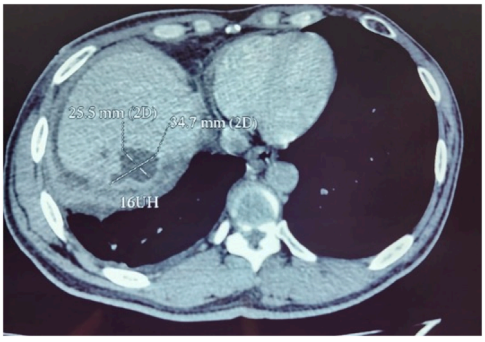

Fig. 3. Contrasted Abdominal computed tomography (CT) revealing residual abscess in segment VII measuring 2.5 × 3.4 cm in size containing less of 10 cc.

Determination of the hypermucoviscous phenotype

The *K. pneumoniae* 14652 isolate was grown onto a MacConkey plate for 18 h at 37 °C [8]. The isolate was negative to the formation of a viscous string.

Whole-genome sequencing and bioinformatic analyses

Total genomic DNA was extracted and purified using the DNeasy Kit (Qiagen, Germany). WGS was generated using Illumina (MiSeq) platform. Evaluation of assembly quality was performed with QUAST (Quality Assessment Tool for Genome Assemblies). Quality-based trimming was performed with the trim galore v.0.4.4 and de novo assembly was done with Unicycler v0.4.9b. The virulome, resistome and MLST were determined by the Kleborate platform [9], and plasmidFinder for plasmid replicon type (http://www.genomicepidemiology.org).

The whole-genome SNP alignment was performed using Snippy v4.6.0 and RAxML was used for constructing the phylogeny under the GTRGAMMA model.

For this analysis we included the hvKp 14320, 14731, 13801, 14313, 14660, 10271, 14682, 14652 and the atypical hvKp 13526 previously described in Mexico. In addition, we included the genomes reference SGH10 and NTUH-K2044 from lineage ST23-K1.

Pathogenicity assay

The pathogenicity of the isolate was evaluated using an *in vivo* experiment. Groups of five healthy male BALB/c mice were obtained from the animal facility of the National Institute of Public Health at the age of 6–7 weeks and weight between 18 and 24 gr. Briefly, bacteria were grown overnight in LB and were subsequently serially diluted to obtain 100–100,000 CFU/ml in 1 × PBS. A 100-µl bacterial suspension was injected intraperitoneally. Mice were monitored twice daily for twelve days post inoculation. For each, experiment five mice injected with sterile PBS were used as negative control and the hv strain 14660 (ST86-K2) was used as control of hypervirulence [5].

Outcomes

The strain was determined to be resistant to ampicillin and tetracycline and sensitive to piperacillin/tazobactam, aztreonam, cefotaxime, ceftazidime, imipenem, gentamicin, amikacin, ciprofloxacin and nalidixic acid (Table 1).

The genome analysis revealed that the *K. pneumoniae* 14652 isolate corresponded to the ST23, capsular locus KL1 and the plasmid replicon types repB/HI1B(pNDM-MAR). The virulence-associated genes corresponded to *rmpADC* (regulators of capsule and hypermucoviscosity production), aerobactin (*iuc*), yersiniabactin (*irp/ybt*), salmochelin (*iro*) and colibactin (*clb*) allele 2; however, this isolate was negative in the string test (Table 1). A whole-genome-SNP phylogeny confirmed a genetic relationship with hypervirulent *K. pneumoniae* K1 reference strains

Table 1
Molecular characteristics and antimicrobial susceptibility profile of the atypical hypervirulent *Klebsiella pneumoniae* K1-ST23.

Isolate	Molecular characteristics								Susceptibility (MIC µg/ml)									
	String test	Serotype	ST	*rmpADC*	*rmpA2*	*iucA*	*ybt*	*clb*	*iro*	AMP	PIP/TAZ	AZM	CTX	CAZ	IMI	GM	AK	CP	AN	TC
K. pneumoniae 14652	-	KL1	23	+	-	+	+; ICEKp10	+	+	R	S	S	S	S	S	S	S	S	S	R

described worldwide and hypervirulent *K. pneumoniae* K2 described in Mexico [9] (Supplementary Figure). Antibiotic susceptibility and molecular tests indicated a pan-susceptible phenotype. The pathogenicity of this isolate was evaluated in mouse model showing 20 % lethality (Fig. 4). Mutations in *rmpADC* genes that could explain the absence of hypermucoviscosity phenotype were searched for, but the genes that make up the *rmp* operon were intact.

Discussion

We described a case compatible with hvKp invasive infection that was confirmed by culture of pus drainage and subsequent molecular species identification. The patient had typical characteristics that are associated with high susceptibility for developing a hvKp infection; these are an Asian origin and recent diagnosis of diabetes mellitus. Intriguingly, the main distinction of hvKp, the hypermucoviscosity, was absent.

Currently, there are significant knowledge gaps that include the route of hvKp entry into humans, ethnic background, and other risk factors [1]. Some studies propose that disruptions of mucosal or epithelial barriers due to the use of medical devices may enable the entry of hvKp in hospitalized patients, but for healthy individuals is unclear how this occurs [1]. Moreover, it has been proposed that hvKp infection occurs more frequently in Asians from regions with high pathogen exposure and increased colonization [1,10]. According to Lee and colleagues, Chinese individuals had higher prevalence of hvKp-K1 infection than the predominantly diabetic non-Chinese (Malays, Indian and Caucasian) and one explanation is that higher intestinal carriage rate occur in the Chinese compared to the non-Chinese [10]. These findings support that ethnic predisposition represent a risk factor for acquiring hvKp and agrees with demographic features of the patient from this case report.

Colonization with hvKp appears to be a requisite for infection in healthy individuals. Although the duration of colonization has not been established, Harada and colleagues reported that carriage of hvKp amongst family members could be maintained for at least 2 years [11]. So, it is possible that the patient from this case report was a long-term carrier of hvKp that acquired the bacteria in his natal country or other Asian country. In addition, diabetes mellitus (DM) appears to be a variable trait amongst patients infected with hvKp; one study found that patients in health care and hospital settings had higher proportions of DM [12] which agrees with the recent diagnosis of the patient reported in this case, but other studies conclude that DM is not a risk factor for developing hvKp infection [5,10].

The *rmpADC* operon is a key driver in capsule regulation and production of hypermucoviscosity [13]. Kochan and colleagues reported

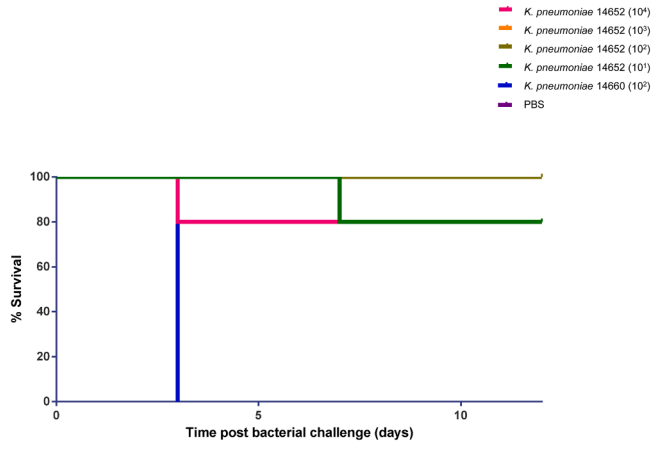

Fig. 4. Survival curves for mice inoculated with four different doses of the hypervirulent *K. pneumoniae* 14652 isolate.

hvKp-ST23 and hvKp-ST16 strains were non-hypermucoviscous [14], and similarly Rodríguez-Medina and colleagues [5] reported hvKp non-hypermucoviscous strains belonging to ST23, ST380 and ST3999. In both studies, despite isolates containing the *rmpADC* operon, mutations occurring in *rmpA* and SNVs upstream of *rmpA* and *rmpD* altered the production of hypermucoviscosity. The strains from both studies were obtained from multiple infection sites such as urine, blood, and eye secretion. This indicates that atypical hvKp is associated with invasive and non-invasive infections. In this case report, the hypervirulent but non-hypermucoviscous *K. pneumoniae* 14652 isolate possessed an intact *rmpA* (data not shown). The lack of the hypermucoviscous phenotype matches with its low *in vivo* virulence despite the presence of genetic and genomic characteristics that are shared with any hv strain. We do not know the cause of hypermucoviscosity loss, and our results are not sufficient for making a hypothesis. This needs to be further evaluated as we may facing the evolution of novel hvKp phenotypes that confer less fitness costs.

Clinicians are not familiar with invasive infections caused by hvKp, thus proper clinical guidelines and diagnostic probes are needed. As mentioned, the string test itself is not an effective way to identify hvKp strains, but its application in the clinic could be complemented with clinical, demographic, and molecular data, such as the amplification of *rmpA* and *iucA* genes; these genes have shown high sensitivity and specificity as markers for identifying hvKp and atypical hvKp [16]. Moreover, Russo and Marr pointed out that the genes *peg-344, iroB, iucA, rmpA, rmpA2* and siderophore production could be part of a promising diagnostic test that could be used by clinical laboratories [1].

In Mexico there is no information about the underlying causes of pyogenic liver abscess, and to date two reports of hvKp-K2 have been described [15,16]. Thus, the disease burden attributable to hvKp in the Mexican population is unknown. This is worrying from a national perspective because infections caused by hvKp are at high risk of becoming severe and requiring a systemic evaluation, and there are no clinical guidelines for diagnosis, treatment, and patient management. Of note, the previous and current report of pyogenic liver abscess in Mexican individuals [15,16] were from patients that received private medical attention; therefore, the information from public health care settings is even more scarce. This represents a field for future investigations.

In summary, we report a case of a Chinese patient who developed an invasive infection caused by an atypical hvKp, that belongs to pandemic clone (ST23-K1). The infection was successfully resolved in part for the non-MDR phenotype and also the absence of hypermucoviscosity could contribute to decrease the tolerance of antibiotics resulting in clearance of hvKp.

CRediT authorship contribution statement

José Juan Donis-Hernández: Conceptualization, Data curation, Formal analysis, Methodology, Supervision. **Ofelia Alma Perez-Rezendiz:** Conceptualization, Data curation, Formal analysis. **Neli Nava-Domínguez:** Formal analysis, Investigation, Methodology. **Luis Duarte-Zambrano:** Formal analysis, Methodology. **Elsa Maria Tamayo-Legorreta:** Investigation, Methodology. **Ulises Garza Ramos:** Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Writing – original draft, Writing – review & editing. **Lucia Martínez-Hernandez:** Conceptualization, Data curation, Investigation, Supervision, Validation, Writing – review & editing. **Alejandro Alvarado-Delgado:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Nadia Rodríguez-Medina:** Formal analysis, Methodology, Software, Writing – review & editing. **Jorge Garcia-Peniche:** Conceptualization, Data curation, Formal analysis.

Ethics approval and consent to participate

Signed informed consent was obtained from patients. All methods were carried out in accordance with the guidelines from the at Instituto Nacional de Salud Pública. This study was approved by the Biosafety and Ethics committees at Instituto Nacional de Salud Pública under the number CB21-025 and CI: 1721, respectively.

Author Statement

The authors have reviewed this version of the document and agree to submit it.

Declaration of Competing Interest

The authors not declared conflict of interest.

Data availability

The data described in this Data note can be freely and openly accessed on the NCBI under the accession number JAWMWE000000000 and biosample SAMN37985424.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.idcr.2024.e01987.

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