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Case report

Hypermucoviscous *Klebsiella pneumoniae*: A challenge in community acquired infection

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ABSTRACT

In 1986, a new syndrome was described in Taiwan secondary to hypervirulent *K. pneumoniae* (hvKP), and its main feature was the ability to cause severe infection in young and immunocompetent hosts. Their virulence is explained by the efficient acquisition of iron and an increase in capsule production, which confer the characteristic hypermucoviscous phenotype. Most of these cases have been described in Asia and subsequently spread to America and Europe, where their prevalence is much lower. We present four cases of bacteremia and liver abscesses secondary to hypervirulent *K. pneumoniae,* two of them associated with endophthalmitis. *K. pneumoniae* isolates recovered from two of the patients belonged to capsular serotype K1 (genes *wzx_K1* and *magA*), while the other two were K2 (gene *wzy_K2*). Both of the K1 isolates were classified into a ST23, and isolates of serotype K2 belonged to the ST375 and ST881 clones.

In Europe, hvKP isolates are less frequently recovered, mostly associated with Asian citizens or travelers, which was not the case in our patients. K1 capsular serotype is a major cause of primary liver abscess and secondary septic embolus, and K2 is associated with secondary liver abscess. Although these hypervirulent variants usually affect immunocompetent patients as in our cases, diabetes mellitus is a major risk factor for the most invasive cases, with concomitant poor prognosis. Identification of hypervirulent *K. pneumoniae* serotypes K1 and K2 should be considered as part of the microbiological diagnosis of community-acquired liver abscess due to their clinical implications.

Introduction

Klebsiella pneumoniae is a Gram-negative bacillus, which can be a member of the indigenous intestinal and nasopharyngeal microbiota in humans. However, it is also responsible for both community acquired and nosocomial infections, such as urinary tract infections, bacteremia, pneumonia and intraabdominal infections, acting as an opportunistic pathogen.

In 1986, Liu et al. described a new syndrome in Taiwan caused by *K. pneumoniae* which consisted in the presence of liver abscess associated with septic endophthalmitis [1]. Almost 20 years later, Fang et al. identified a novel virulence gene, *magA*, in *K. pneumoniae* isolates causing primary liver abscess and septic metastatic complications [2]. These authors also found that the hypermucoviscous phenotype (defined as the ability of forming a viscous string >5 mm in length) was significantly associated with *K. pneumoniae* invasive isolates.

This new organism was identified as hypervirulent *K. pneumoniae* (hvKP), and its main feature was the ability to cause severe infection in young and immunocompetent hosts. These hvKP are responsible for a syndrome that includes pyogenic liver abscess and metastatic spread to eyes, central nervous system and lungs. First in Asia, and then in America, Africa, Australia and Europe, an increasing number of infections caused by this organism are being reported [3].

The majority of these hvKP belong to capsular serotypes K1 and K2. Their virulence seems to be explained by the efficient acquisition of iron and an increase in capsule production, which confer the characteristic hypermucoviscous phenotype.

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Case reports

We present clinical and microbiological features of four cases of invasive infection in Caucasian patients without relevant epidemiological history, caused by two different serotypes of hvKP (K1 and K2) in an 1160 beds tertiary hospital in Madrid (Spain):

- **Patient 1:** An 86-year-old female with a history of hypertension and atrial fibrillation attended the emergency room with 3-day fever, ocular pain and decreased vision in her right eye. Endogenous endophthalmitis was suspected. The patient was hospitalized and intravitreous vancomycin and ceftazidime treatment was administered. After admission, abdominal Computed Tomography (CT) showed hepatic abscesses involving the right lobe of the liver, which were drained. *K. pneumoniae* was isolated from blood cultures, liver abscess, urine culture and vitreous fluid cultures. Intravenous cefotaxime (2 g every 4 h) was administered for 4 weeks. After completing the antimicrobial treatment, the liver abscess was resolved but her vision remained diminished.
- **Patient 2**:A 72-year-old male with chronic hepatopathy due to hepatitis C virus attended the emergency room with fever (38.5 °C) and pain in the right hypochondrium. Abdominal CT-scan showed an abscess of 10 cm in diameter in the right hepatic lobe. Drainage was performed, obtaining purulent material that was sent for culture. *K. pneumoniae* was obtained from blood and liver abscess cultures. The patient received ceftriaxone 2 g every 24 h iv during 4 weeks, achieving complete recovery.
- **Patient 3:**A 57-year-old male with primary hyperaldosteronism attended the emergency room with fever (39 °C), cough and pleuritic chest pain, treated empirically with ceftriaxone. An infiltrate in the right upper lobe with nodular opacities was observed in his chest x-ray. Immediately after admission, he began to present ocular pain and decreased vision in his right eye. After diagnosis of endogenous endophthalmitis, intravitreous treatment with vancomycin, ceftazidime and corticosteroids was administered. Abdominal CT-scan showed two hepatic abscesses involving the right lobe. Drainaintravenous cefotaxime (2 g every 4 h) was administered. *K. pneumoniae* was recovered from blood and liver abscess cultures. After 4 weeks of antimicrobial treatment, improvement of the hepatic abscesses and total recovery of vision was achieved.
- **Patient 4:** An 82-year-old male with a history of Parkinson's disease, chronic pancreatitis and hypertension attended the emergency room with fever (40 °C), deterioration of general condition, abdominal pain, dysuria and frequency. An abdominal CT-scan showed findings suggestive of cholangitis and multiloculated liver abscess in the left lobe. Drainage of the lesion was not performed due to expectation of limited yield when a loculated abscess is present. *K. pneumoniae* was recovered from blood cultures. The patient initially received meropenem (1 g every 8 h) and linezolid (600 mg every 12 h) and after four days, was de-escalated to intravenous ceftriaxone (2 g every 24 h). He completed 3 weeks, followed by 3 weeks of oral cefixime. Complete recovery was achieved.

Microbiological data

Identification (ID) and antimicrobial susceptibility testing (AST) following EUCAST criteria (EUCAST breakpoint version 9.0, www.eucast.org) were carried out by the automated system MicroScan® Walkaway (Beckman Coulter, West Sacramento, CA, United States) using negative combo panel type 53. All IDs were confirmed by MALDI-TOF Microflex Biotyper (Bruker Daltonics, Bremen, Germany).

In order to determine the capsular serotype K1 and K2, polymerase chain reactions (PCRs) were performed using specific primers for the detection of the genes *wzx_K1*,*wzy_K2* and *magA* [4]. Genetic material from *K. pneumoniae* isolates was extracted using commercial kit QIAamp DNA Mini Kit (Qiagen, Venlo, The Netherlands). Pulsed-field ground electrophoresis (PFGE) was performed according to PulseNet PFGE protocol for *E. coli* O157:H7, *E. coli* non-O157 (STEC), *Salmonella*, *Shigella sonnei* and *Shigella flexneri* to study genetic relatedness [5]. To analyze the clonality of the specimens a multilocus sequence typing (MLST) scheme was performed following the protocol described by Diancourt et al [6] and the alleles and sequence types (STs) were assigned according to the MLST international scheme of the Institut Pasteur, Paris, France (http://bigsdb.pasteur.fr/perl/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef_public).

K. pneumoniae isolates recovered from patient 1 and 2, belonged to capsular serotype K1 (genes *wzx_K1* and *magA*) while the other two (patient 3 and 4) were K2 (gene *wzy_K2*). Both of the K1 isolates were classified as ST23, which is one of the major clonal complexes that includes only K1 isolates, and has been described as more virulent than other STs according to *in-vitro* studies [7]. These ST23 strains were not related by PFGE, indicating that a specific clone may not cause *K. pneumoniae* liver abscess. *K. pneumoniae* isolates of serotype K2 belonged to the ST375 and ST881 clones.

The hypermucoviscous phenotype can be measured semiquantitatively by the "string test", which consists in the formation of a viscous string >5 mm in length when bacterial colonies on an agar plate are stretched by an inoculation loop. All isolates were positive for this test (Fig. 1). Both serotypes were highly susceptible to all antimicrobials tested including amoxicillin/clavulanate, extended spectrum cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, rifampin, and colistin.

Discussion

Since 1980, especially in Southeast Asia, there has been an increase in the number of reported cases of community infections due to *K. pneumoniae* specifically by hypermucoviscous strains causing liver abscesses [2,8]. In Europe, K1 and K2 *K. pneumoniae* isolates are less frequently recovered, and some cases have been associated with Asian citizens or travelers, which was not the case in our patients [3,9].

K1 capsular serotype is a major cause of primary liver abscess and secondary septic embolus and K2 is related to secondary liver abscess. The expression of these capsular serotype antigens confers a mayor resistance to phagocytosis. The gen *magA* in K1 provides the formation of a protective exopolysaccharide associated with

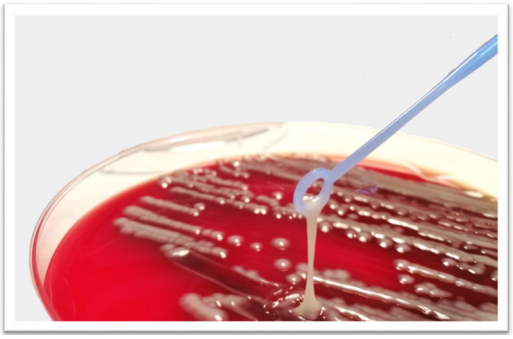

Fig. 1. "String Test" confirming the hypermucoviscosity of a *K. pneumoniae* isolate recovered from blood culture (patient 4). A positive result is defined as the formation of a viscous rope greater than 5 mm when a bacterial colony is touched with a loop on an agar plate.

hypermucoviscosity, and the capacity to develop liver abscess and septic metastasis. Moreover aerobactine, an iron chelating siderophore, present in these isolates, potentiates the virulence of hvKP, as has been demonstrated in animal models [8]. In routine laboratory practice, the string test is a useful screening method for subsequent molecular characterization in order to detect possible hypervirulent strains (Fig. 1). Although these hypervirulent variants can affect immunocompetent patients as in the cases presented herein, diabetes mellitus seems to be a major risk factor for the most invasive cases with concomitant poor prognosis, especially in cases of endophthalmitis [10].

Because of the potential for causing metastatic infections, clinicians should be aware of the possibility of serious complications. Among others, it has been suggested that strict glycemic control could help to avoid the development of septic metastatic complications, but more clinical studies are still necessary to definitively suggest the benefit of strict glucose control in these cases [11]. Lungs, central nervous system, and eyes are the most common metastatic sites, but only a third of them are diagnosed on admission. Meningitis and endophthalmitis are associated with poorer outcome in 10–12% of cases, and meningitis is associated with high mortality rates [1,12]. Prognosis for patients with endophthalmitis caused by *K. pneumoniae* is very poor; more than 85% of patients had a severe visual deficit, however prognosis improves with early diagnosis and early intravenous and intravitreal treatment [13].

Finally, hvKP strains usually show high antimicrobial susceptibility, but this pattern seems to be decreasing over time [14]. Selection of antimicrobial treatment should be guided by in-vitro AST. Adequate drainage of abscesses is recommended for better clinical response. Three weeks of treatment has been recommended, as shorter courses of treatment are responsible for a high rate of relapses.

Conclusions

Outside Asian countries, isolation of hypermucoviscous *K. pneumoniae* isolates is uncommon. However, the incidence is growing, and is being isolated in individuals without the usual risk factors (diabetes mellitus or Asian origin) associated with infection due to these types of strains. Unlike some recently reported cases, our isolates are of community origin and present with high antimicrobial susceptibility. It is important to improve detection of hvKP isolates in order to optimize outcomes and avoid potential metastatic complications.

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Conflict of interest

Authors have no conflicts of interest to declare with respect to the contents of this manuscript.

CRediT authorship contribution statement

Javier Sánchez-López: Conceptualization, Writing - original draft, Formal analysis, Investigation. **Andrea García-Caballero**: Conceptualization, Writing - original draft, Formal analysis, Investigation. **Carolina [45_TD$DIFF]Navarro-San Francisco**: Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Project administration. **Carmen Quereda**: Resources, Writing - review & editing. **Patricia Ruiz-Garbajosa**: Validation, Writing - review & editing, Resources. **Enrique Navas**: Resources, Writing - review & editing. **Fernando Dronda**: Resources, Writing - review & editing. **María Isabel Morosini**: Conceptualization, Validation, Writing - review & editing. **Rafael Cantón**: Writing - review & editing, Supervision, Funding acquisition. **María Diez-Aguilar**: Conceptualization, Writing - original draft, Writing - review & editing.

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