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Review article

Hypervirulent *Klebsiella pneumoniae*: Epidemiology outside Asian countries, antibiotic resistance association, methods of detection and clinical management



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ABSTRACT

Two main *Klebsiella pneumoniae* pathotypes are of public health concern, classical *K. pneumoniae* (cKP), with high antibiotic resistance acquisition capacity, and hypervirulent *K. pneumoniae* (hvKP). The emergence of hypervirulent and antibiotic-resistant *K. pneumoniae*, especially carbapenem resistance, is worrisome and require effective methods for detection and treatment. Different evolutionary paths contribute to the emergence of hypervirulence and antibiotic resistance, commonly via the acquisition of resistance plasmids by hvKP (CR-hvKP) or the acquisition of virulence plasmids by CRKP (hv-CRKP). ST11-KL64 together with *bla*_{KPC-2}, is the most extended hv-CRKP lineage acquiring virulence plasmids with associated biomarkers, *rmpA*, *rmpA2*, *iroBCDEN*, *iucABCDiutA*, and *peg344*. In addition to ST11, other hv-CRKP clones have been reported in Europe such as ST101, ST147 and ST512, highlighting the association of ST147 with OXA-48 and NDM carbapenemases. Although still very rare in Spain, hvKP cases are increasing in recent years, mainly due to ST23-K1, ST380-K2 and ST86-K2. Management of hvKP infections requires active antibiotic therapy based primarily on antibiotic susceptibility patterns and site of infection.

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Klebsiella pneumoniae hipervirulenta: epidemiología en países no asiáticos, asociación con la resistencia a antibióticos, detección y manejo clínico

RESUMEN

Existen 2 patotipos de *Klebsiella pneumoniae* con impacto en salud pública: *K. pneumoniae* clásica, con alta capacidad de adquisición de resistencia a antibióticos, y *K. pneumoniae* hipervirulenta (hvKP). La aparición de *K. pneumoniae* hipervirulenta y resistente a antibióticos es preocupante y requiere de métodos efectivos para su detección y tratamiento. Dos vías evolutivas principales contribuyen a la aparición de cepas hipervirulentas y resistentes a carbapenémicos: la adquisición de plásmidos de resistencia por hvKP (CR-hvKP) o la adquisición de plásmidos de virulencia por CRKP (hv-CRKP). ST11-KL64 productora de KPC-2 es el linaje hv-CRKP más extendido, con plásmidos portadores de genes de virulencia: *rmpA*, *rmpA2*, *iroBCDEN*, *iucABCDiutA* y *peg344*. Otros secuenciotipos de hv-CRKP descritos en Europa son ST101, ST147 y ST512, destacando la asociación de ST147 con OXA-48 y NDM. Aunque es infrecuente en España, los casos de hvKP están aumentando en los últimos años, principalmente debido a ST23-K1, ST380-K2 y ST86-K2. El manejo de infecciones por hvKP requiere tener en cuenta principalmente la sensibilidad antibiótica y el sitio de la infección.

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Introduction

Klebsiella pneumoniae is an opportunistic pathogen capable of causing severe and life-threatening infections such as pneumonia, bacteraemia and complicated urinary tract infections.^{1,2} *K. pneumoniae* has the ability to acquire new genetic material, which has in fact enabled its differentiation into two circulating pathotypes termed classical *K. pneumoniae* (cKP) and hypervirulent *K. pneumoniae* (hvKP).^{2,3} These pathotypes present clinical, geographical and molecular differences. cKP isolates usually produce nosocomial infections in immunocompromised patients or those with underlying diseases, and they have increasingly acquired antibiotic resistance determinants, mainly carbapenemases,^{3,4} representing a 'critical concern' according to the World Health Organization.⁵ On the other hand, in the last decades hvKP has emerged as a virulent pathogen capable of causing invasive community acquired infections in healthy individuals. These infections are characterized by a rapid metastatic spread and generation of pyogenic tissue abscesses.^{2,4} Until recently, each pathotype had shown a predominant geographical distribution, mainly limited to the Asian Pacific Rim countries in the case of hvKP, or to the Western countries for cKP.^{1–4}

HvKP was initially identified as a cause of pyogenic liver abscesses and severe endophthalmitis in Asia,⁶ and later recognized as an entity more virulent than cKP. Although the most common clinical syndrome caused by hvKP is liver abscess without underlying hepatic disease, it can cause infections in other organs or systems such as pneumonia, necrotizing fasciitis, endophthalmitis, meningitis and non-hepatic abscesses.²

The hypermucoviscous phenotype is a frequent feature of hvKP that is related to the *magA* and *rmpA* virulence genes and can be detected by the string test.^{1–4} However, the presence of this phenotype is not fully defining hvKP and additional virulence markers are required. The biomarkers that have demonstrated a better accuracy (>95%) to differentiate hvKP and cKP isolates are *peg-344* (putative transporter), *iroB* and *iucA* (siderophores) and plasmid-borne *rmpA* and *rmpA2* genes (regulators of the mucoid phenotype via increased capsule production), while the string test or K1 and K2 capsule types, frequently associated to hvKP, presented a lower accuracy.⁷

Early reports of hvKP infections were mainly due to susceptible isolates belonging to clonal complex (CC)23 and K1 capsular type in China and other Asian Pacific Rim countries. The prevalence of hvKP varies widely by geographic regions, ranging from 12% to 45% in Asian hvKP-endemic areas.^{1–4} However, the epidemiology of hvKP is changing rapidly, becoming more complex and varied. Both, worldwide dissemination of hvKP infections, and the association of multi-drug resistance (MDR) with this pathotype are increasingly being reported in the last years.^{1–4} The differentiation between both pathotypes is becoming blurred due to two fundamental causes: (i) the capacity of K1/K2 hvKP clones to acquire plasmids that confer MDR (CR-hvKP), and (ii) the possibility that MDR high-risk clones of cKP, such as ST11, ST147 or ST307, take up virulence plasmids (hv-CRKP).^{1–4}

In this systematic review, we aimed at summarizing recent information on the hvKP epidemiology outside Asia, the association between hypervirulence and antibiotic resistance, and diagnostic methods.

Material and methods

The objective of this review was to gather novel and relevant studies that has been published in recent years on hypervirulent *K. pneumoniae* strains, focusing on their epidemiological impact outside Asia, diagnostic methods as well as their association with

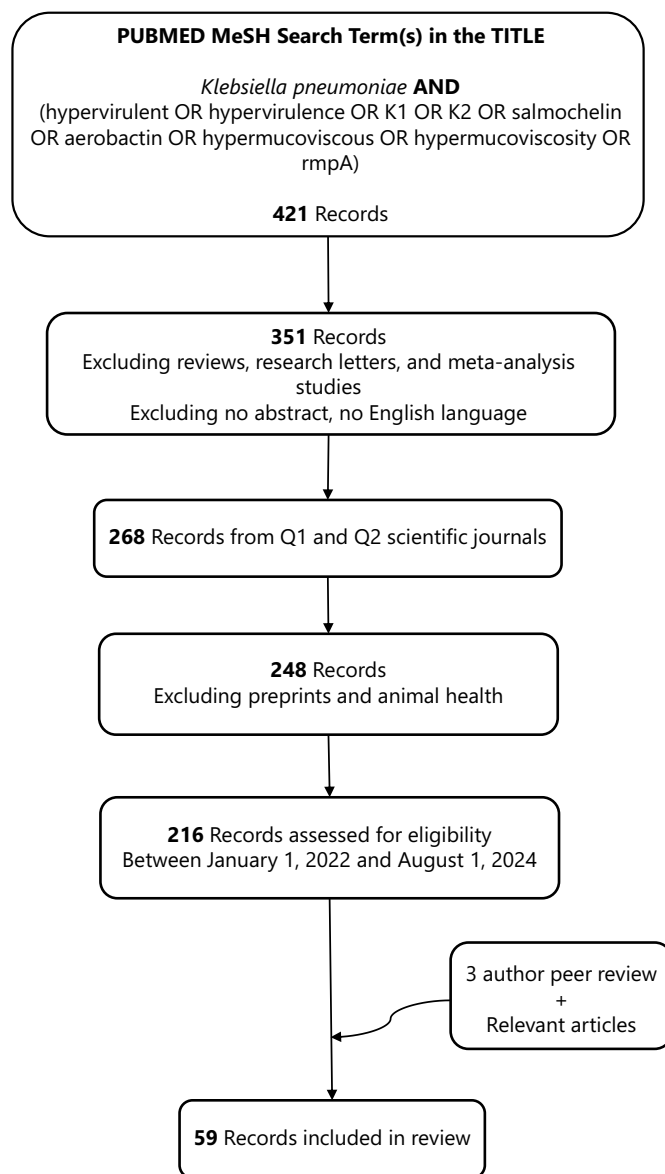


Fig. 1. PRISMA flow diagram of scientific articles included in this review.

antibiotic resistance. The systematic review conducted for this study was adhered to the PRISMA guidelines.⁸

Publications available in PubMed database from January 1, 2022 to August 1, 2024 were reviewed. The searching criteria included the following combination of terms in the title: *K. pneumoniae* AND (hypervirulent OR hypervirulence OR K1 OR K2 OR salmochelin OR aerobactin OR hypermucoviscous OR hypermucoviscosity OR *rmpA*). A total of 421 records were retrieved. Research letters, reviews, meta-analysis and preprints were excluded. We identified articles that included abstract and were published in English language in Q1 and Q2 scientific journals, and only publications on humans were considered (Fig. 1).

A total of 216 records were assessed for eligibility. All title and abstracts were screened by three authors (JO-I, MP-V and SG-C) for possibly eligibility. The eligibility criteria varied according to the objectives: (1) for revision of phenotypic and genotypic diagnostic methods, mobile genetic elements of transmission, associated antimicrobial resistance and clinical management, articles from all geographical origins and specific relevant reviews were considered; (2) for epidemiological purposes, articles from Asian

countries were excluded, since this region is associated with a high and well-known incidence of these strains. A total of 59 articles were included.

Results and Discussion

Epidemiology and population structure of hypervirulent K. pneumoniae in non-Asian countries

The analysis of the hvKP/CR-hvKP population showed that the most common lineage associated with these pathotypes is clonal group (CG)23 (including ST23, 25, 57 and 1633), followed by CG65 (including ST65 and 375) and CG86.⁹ Additionally, capsular types commonly associated to hvKP/CR-hvKP are, in order of frequency, K1, K2, and K57.⁴ In the case of hv-CRKP, the most frequent carbapenem-resistant or MDR clones acquiring virulence plasmids belonged to ST11, KL64 or KL47.¹⁰

First descriptions of hypervirulent strains involved mostly South and Southeast Asia, but the number of reports of infections attributed to hvKP isolates has increased worldwide. In European countries, the majority of hvKP reports are sporadic cases, describing retrospective collections of isolates. The most frequent combination of ST and K-type is ST23-K1, detected in Germany, Italy, Switzerland and Poland,^{11–14} being in most cases associated with a high virulence score, according to the presence of key loci related to increasing virulence (yersiniabactin < colibactin < aerobactin) and following Lam et al. classification.¹⁵ ST23 has also been described in combination to less frequent K-types, such as K57 and KL107.¹⁴ Recently, the emergence of ST23 associated with carbapenemase production in Europe, led to a public health alarm raised by the European Centre for Disease Prevention and Control (ECDC).¹⁶ In addition, other less frequent STs associated with hpKP, ST86, ST5, ST8, ST25 and ST6310, have been described in Italy.^{13,17}

Regarding hv-CRKP population, the ST395-K2 high-risk clone commonly associated with MDR, has been detected in Germany, Italy and Switzerland, producing different types of carbapenemases, KPC-3, NDM-1 and OXA-48.¹² Other hv-CRKP clones such as ST101, ST147 and ST512 have been reported in Europe.¹³ These STs have been involved in outbreaks in some cases, such as a ST147 outbreak in patients with SARS-CoV-2 pneumonia in Italy.¹⁸

Since the first description of hvKP in United States, the incidence of hvKP infections has increased. Recent surveillance studies showed that in North America, similar to Asia and Europe, the most frequent hvKP detected was ST23-K1, but in comparison to Asia, a larger genetic diversity was detected, including K2 lineages combined with different STs: ST66, ST390 and ST375.^{19,20}

Cases of hvKP have been sporadically reported in South America in different countries such as Argentina, Brazil, Chile and Mexico. These cases belonged to the same lineages described all around the world, ST23-K1 and ST86-K2. Other clones like ST1161-K19 have been only detected in Chile and Brazil, suggesting that this serotype could be endemic to South America.²¹

In Africa, information on the newly emerging hvKP lineages is scarce, although a study carried out over a period of 12 months (September 2020–October 2021) in a tertiary hospital in South Africa, detected the following lineages: ST20-K1, ST23-K2, ST65-KL28, ST985-KL39 and ST3430-KL52.²² Combination of MDR and hypervirulence has emerged in different countries such as Egypt, Nigeria and Ghana, being ST11-KL47 and ST147-K67 the lineages detected, with a suspicion of cross border transmission in some cases.²³

A few studies in Spain describe hypervirulent *K. pneumoniae* strains. Cubero et al. reported bacteremia episodes due to hypermucoviscous *K. pneumoniae*, detecting as the most frequent clones;

ST23-K1, ST380-K2 and ST86-K2.²⁴ Other Spanish study reported ST23-K1 and *bla*_{OXA-48}-producing isolates.²⁵

In the last 5 years the Spanish National Center of Microbiology, via its Surveillance Program on Antibiotic Resistance, has received a total of twelve hvKP clinical strains from Spanish hospitals (unpublished data). The ST23 was predominant, followed by ST380, and all isolates belonged to K1 and K2 types. In addition, an outbreak by hypervirulent VIM-producing ST23-K1 strains, two infection cases and one colonization, has been recently reported in Gran Canaria (Canary Islands) (del Rosario Quintana C. et al., unpublished data).

Antibiotic resistance in hypervirulent K. pneumoniae

Hypervirulent and carbapenem-resistant K. pneumoniae

Three different evolutionary paths have contributed to the emergence of hypervirulent and carbapenem-resistant *K. pneumoniae* isolates: (i) hvKp strains acquire carbapenem-resistance plasmids (CR-hvKP); (ii) carbapenem resistant cKP acquire virulence plasmids usually harboring pLVPK-associated markers (*rmpA*, *rmpA2*, *iroBCDEN*, *iucABCDiutA*, and *peg-344*) (hv-CRKP); and the less common, (iii) the convergence of virulence and carbapenem resistance genes in a single plasmid, acquired by hvKp or CRKP strains.²⁶

CRKP belonging to ST11 is the most common sequence type acquiring hypervirulent determinants.^{10,18,27–32} A phylogenetic evaluation performed with more than 1800 *K. pneumoniae* genomes from China and assemblies from public databases revealed pk2044-like virulence plasmids in ST11-KL64 derived from ST23-K1 hypervirulent *K. pneumoniae*. The identified virulence genes in these plasmids were *rmpA*, *rmpA2*, *iroN*, *iucA*, or *pagO*, with a deletion improving survival of hv-CRKP in macrophages.³² A previous study in elderly patients with CRKP infections in the intensive care unit (ICU), also reported a strong association between hypervirulence-CRKP (63.8%, 51/80) and ST11-KL64. Five virulence-associated genes were described in this study, being the most common *rmpA2*, followed by *iucA*, *iroN*, *peg-344*, and *rmpA*.³³

The main mechanism of carbapenem resistance described in hv-CRKP ST11 is the presence of the *bla*_{KPC-2} gene, followed by *bla*_{NDM-1} and *bla*_{OXA-48}.^{28,34,35} Some studies have reported cases of carbapenemase co-production, although in different plasmids, i.e. *bla*_{KPC-2} and *bla*_{NDM-1} genes in one isolate from a tertiary hospital in Wuhan,²⁷ or *bla*_{KPC-2} and *bla*_{OXA-48} genes in one isolate from Egypt.¹⁰ On the other hand, hypervirulent high-risk clones ST11-K2 and ST15-K54 harboring *bla*_{NDM-1} (CR-hvKP) on conjugative plasmids, have been described causing neonatal sepsis. Two of these isolates, ST11 and ST15, also had pLVPK-associated markers.²⁹

A systemic retrospective study conducted in a Chinese tertiary hospital on *K. pneumoniae* ST147, revealed six isolates, two of them MDR KL64 and hypervirulent by acquiring *bla*_{OXA-48} and key virulence genes, *iucA+rmpA2*. A fusion plasmid encoding virulence genes, *iucABCDiutA*, *peg-589* and *rmpA2*, and antibiotic resistance gene, *sul2* was detected in both isolates, likely generated by recombination of a plasmid like KpvST101.OXA-48 with the classic virulence plasmid of hvKp via an IS26. In addition to the fusion plasmid, a MDR IncL/M plasmid replicon type, harboring *bla*_{OXA-48}, *bla*_{CTX-M-16}, *strA* and *strB*, was identified.³⁶

Hypervirulent and antibiotic-resistant K. pneumoniae other than carbapenems

A novel KPC-2 variant, KPC-135, conferring resistance to ceftazidime–avibactam has been described in a ST11-K47 hypervirulent *K. pneumoniae* clinical strain. This isolate simultaneously had a virulence plasmid harboring *rmpA2* gene.³⁰ Li et al. studied the in vitro activity of ceftazidime–avibactam (CZA), imipenem–relebactam (IMR) and aztreonam–avibactam (AZA)

toward CR-hvKP: ST11-KL64 as predominant type, and *bla*_{KPC-2} and *bla*_{NDM-1} as predominant carbapenemases. The susceptibility rates of CR-hvKP strains to IMR and AZA were lower – 71.9% and 75.0%, respectively – than classical CRKP – 92.5% and 89.5%, respectively – but the molecular antibiotic resistance mechanism was not investigated.³⁷ A recent study evaluated the in vitro activity of cefiderocol, a siderophore cephalosporin, against hvKP. The authors concluded that cefiderocol might be less effective against CR-hvKP, in either NDM-1 or KPC-2-producing isolates, compared with classical CRKP.³⁸

Polymyxin-resistant hvKP, although rare, has become a new superbug prevalent in China. Tang et al. described clonal transmission of ST11-KL64 resistant to polymyxin B due to mutations in chromosomal genes *phoQ* and *pmrB*, and insertion mutations in *mgrB* in a Chinese teaching hospital. Four of these strains co-harbored *bla*_{KPC-2} and *bla*_{NDM-1}, and the plasmid virulence-associated genes *rmpA*, *rmpA2*, *iucA*, and *peg344*.³¹ Regarding mobile colistin resistance (*mcr*), an *mcr*-8.2-harboring hvKP ST412 was identified from pediatric sepsis in a comparative genomic survey in China. This isolate had a pLVPK-like virulent plasmid, encoding siderophores (including aerobactin-encoded gene *iutA*-*iucABCD* and salmochelin-encoded gene *iroBCDN*) and polysaccharide virulence genes (*rmpA* and *rmpA2*).³⁹ Another study reported a ST5571-K2 hvKP co-producing *mcr*-1 and *bla*_{NDM-1}, located in two self-conjugative epidemic plasmids, causing bacteraemia in China and harboring *rmpA* virulence gene.⁴⁰

Hybrid plasmids encoding antibiotic resistance and virulence genes

Hv-CRKP either carry more commonly both virulence and resistance plasmids or carry a large hybrid plasmid coding for both virulence and resistance determinants. A hybrid plasmid IncFIB-IncHI1B harboring virulence genes (*rmpA2*, *iutA*, and *iucABCD*) together with antibiotic resistance genes (*aadA2*, *armA*, *bla*_{OXA-1}, *msrE*, *mphE*, *sul1* and *dfrA14*) was described in a hvKP ST2096 strain in India.⁴¹

In ST11-KL64, with a major association with CR-hvKP, hybrid plasmids have been described. Zhang et al. described a plasmid fusion via an IS26 element, a IncFIB/IncHI1B/IncX3 conjugative plasmid that was also a *rmpA2*-associated virulence plasmid with an *iutA*-*iucABCD* gene cluster.⁴² Two novel hybrid virulence plasmids were also described in ST11-KL64 clinical isolates from China. The IncHI1B/repB-type plasmid co-harboring *bla*_{KPC-2} and *rmpA2*, *iucABCD* and *iutA* virulence genes; and the IncFII/IncR-type virulence plasmid resulted from the recombination between a typical pLVPK-like virulence plasmid, harboring *iutA*-*iucABCD*, *rmpA*, *rmpA2*, and *peg344* genes, and a multidrug-resistance plasmid harboring beta-lactamase genes *bla*_{TEM-1B} and *bla*_{CTX-M-65}, aminoglycoside resistance gene *rmtB1*, and phosphonic acids resistance gene *fosA3*.³⁵

The dominance of pKPC2 plasmid type in clinical settings in Singapore, led to investigate its transmissibility and stability in hypervirulent *K. pneumoniae*. Plasmid conjugation was easy and independent of capsular types. In addition, plasmid sequence was stable and unchanged after moving into different bacterial hosts or even when maintained in human hosts for days.⁴³ Another study on the prevalence of hv-CRKP and CR-hvKP and their evolution factors, revealed that hv-CRKP were more prevalent than CR-hvKP and both were dominated by *bla*_{KPC-2} gene, among public *K. pneumoniae* genomes and a collection of isolates from Chinese hospitals. The conserved *oriT* of virulence plasmids and the widespread of conjugative helper plasmids, such as *bla*_{KPC-2}-carrying plasmids, could mobilize non-conjugative virulence plasmids from hvKP strains to CRKP strains, contributing to the emergence of hv-CRKP strains.²⁶

Hybrid plasmids have also been described in STs other than ST11. A hypervirulent *K. pneumoniae* sublineage (SL)218 (ST23-

KL57) transmitted between hospitalized patients in Denmark, carried a hybrid resistance and virulence repB plasmid containing *iucABCD*, *rmpA*, *rmpA2* and *bla*_{NDM-1}, in addition to other antibiotic resistance determinants: *aph*(3')-VI, *qnrS*, *sul1*, *sul2*, *dfrA5*, *mph*(E), *msr*(E), *mph*(A).⁴⁴ Three Qatari ST383 isolates were reported with a *bla*_{NDM}-like located on an IncHI1B-type plasmid which co-harbored several virulence factors, including the regulators of the mucoid phenotype (*rmpA*, *rmpA2*) and aerobactin (*iucABCD* and *iutA*), likely resulting from recombination events.⁴⁵

Bolourchi et al. investigated plasmid-mediated mediators of hypervirulence using the complete sequence of 79 non-redundant hypervirulent plasmids retrieved from GenBank. The majority of hypervirulent plasmids belonged to clonal complex (CC)23, and ST11, IncFIB and IncHI1B were the most prevalent plasmid replicon types. Almost 99% of plasmids (78/79) had *iutA* and *iucA* genes, and 97% (77/79) had *iucC*, *iucB* and *iucD* genes, and 30% of plasmids carried different antibiotic resistance genes against extended-spectrum beta-lactams, quinolones, aminoglycosides, chloramphenicol, tetracycline and macrolides.⁴⁶

ST383, ST147 and ST15 hypervirulent *K. pneumoniae* isolates were isolated in 2019 in Italy, one of them, ST383, carrying three different plasmids associated with *bla*_{OXA-48}, *bla*_{NDM-1}, *bla*_{NDM-5}. The latter one being a hybrid IncFIB/IncHI1B plasmid harboring also virulence genes, *iucABCD*, *iutA*, *rmpA*, *rmpA2*.⁴⁷

Outer membrane vesicles (OMVs) have been described as novel way for the dissemination of carbapenem-resistant hypervirulent *K. pneumoniae*. Transformation assays have demonstrated that OMVs derived from *K. pneumoniae* could mediate the intraspecific and interspecific horizontal transfer of virulence and resistance plasmids. After isolation and purification of OMVs, identification of *rmpA*, *rmpA2*, *bla*_{KPC-2} was done in OMVs by PCR assay.⁴⁸ Furthermore, co-transmission of virulence (*rmpA2*) and antibiotic-resistance (*bla*_{NDM-1}) genes has been proved via OMV-mediated transformation experiments.⁴⁹

Detecting hypervirulent *K. pneumoniae* in the laboratory

The spread of hvKP, characterized by invasive infections, and its association with carbapenem resistance makes it an emerging threat for public health that requires efficient detection for clinical management, surveillance and outbreak containment. However, clinical laboratories lack an efficient and specific method to differentiate between cKP and the large variety of hvKP strains, since the definition of hypervirulence is even inconsistent. Phenotypically, hypermucoviscous or hypermucoid *K. pneumoniae* strains have been considered hvKP, using the 'string test' as a phenotypic test for hypermucoviscosity, being considered positive when a mucous bacterial colony can be stretched > 5 mm in length with an inoculation loop.⁴ However, not all hypermucoviscous bacteria lead to an invasive syndrome and not all hypervirulent *K. pneumoniae* are hypermucoviscous.⁴

Several methods have been described to identify biomarkers associated with hypervirulence: regulators of the mucoid phenotype (*rmpA* and *rmpA2*), aerobactin (*iucABCD* and *iutA*), salmochelin (*iroB*) and a metabolite transporter (*peg344*). These virulence markers are linked to the hvKP-specific virulence plasmid pLVPK and have shown high accuracy for differentiating between hvKP and cKP strains.⁷ A recent study established hvKP identification criteria as a positive result for any of the above genes or a siderophore yield greater than 30 µg/ml.⁵⁰ Additionally, these authors compared results between CR-hvKP and carbapenem-susceptible (CS)-hvKP regarding these parameters and observed statistical differences ($P < 0.05$). They found a detection rate of *rmpA2*, *iuc* and siderophores production (>30 µg/ml) higher in CR-hvKP than in CS-hvKP, whereas the detection rate of *peg*-344, *rmpA* and *iroB* virulence genes was lower in CS-hvKP than in CR-hvKP.⁵⁰

Table 1
Management and treatment of hvKP.^{2,4,58,59}

Source control	Drainage of liver abscess. Surgical intervention in cases of refractory hvKP infections or infections occurring in critical sites (i.e. brain).	
Empiric antibiotic therapy	Clinical syndrome or site of infection	Empiric antibiotic treatments options (It should be modified according to in vitro susceptibility testing results)
	Liver (or other intra-abdominal) abscess or pneumonia	β-Lactam/β-lactamase inhibitors, third-generation cephalosporins, fluoroquinolones, carbapenems (reserved for extended-spectrum-β-lactamase (ESBL) positive isolates) or aminoglycosides (in combination therapy)
	Central nervous system infection	Third-generation cephalosporins (i.e. ceftriaxone), or carbapenems (i.e. meropenem) for ESBL positive isolates
	Endophthalmitis	Combination of intravitreal antibiotics (i.e., cefazolin, ceftazidime, aminoglycosides or imipenem) and intravenous antibiotics (variable, but usually cephalosporins)
	Prostate abscess	Fluoroquinolones, trimethoprim-sulfamethoxazole, or fosfomycin
Infection control	Implementation of point-of-care testing to identify hvKP in hospitalized patients. Evaluation of infection control measures for patients infected or colonized with antimicrobial resistant hvKP strains, especially carbapenem resistant: contact precautions, informing receiving facilities of patient transfer, and cleaning of areas that the patient has been in contact with on a daily basis.	

Xu et al. developed a multiplex quantitative real-time PCR (qRT-PCR) assay for the simultaneous identification of hvKP and CR-KP. The qRT-PCR included *gltA* gene for *K. pneumoniae* identification, the *iucA*, *rmpA* and *rmpA2* genes for hvKP, and the *bla_{KPC}* gene for CR-KP. The assay had excellent sensitivity and specificity, with regression coefficients (R^2) values above 0.99 for all the four targets using different concentrations of DNA standards.⁵¹ On the other hand, Russo et al. discussed that the absence of some of these markers may reflect the presence of an incomplete virulence plasmid, eventually affecting the hypervirulence phenotype. The authors used both logistic regression and a machine-learning-based prediction model using a classification and regression tree (CART) to determine which variable among biomarker number, total siderophore production, mucoviscosity, Kleborate virulence score, and Mash/Jaccard distances to the canonical pLVPK, was most predictive of the strain cohorts, hvKP and cKP. They concluded that the biomarker count alone was the strongest predictor for both analyses, biomarker count = 5 in the CART model, showing a sensitivity for predicting hvKP of 94% and a specificity of 94%.⁵²

Recombinase-aided amplification (RAA) is a detection method based on RPA (recombinase-based isothermal amplification assay–recombinase polymerase amplification), which has replaced traditional PCR approaches. A recent study established a novel RAA assay targeting *peg34* and *rmpA* for the rapid detection of hvKP in clinical specimens. A combination of recombinases from bacteriophage T4 (key components of RPA) and recombinant enzymes (in RAA) can bind tightly to primer DNA at room temperature. Thus, the RAA assay can be performed at a constant temperature range of approximate 39 °C, with results in maximum 20 min, allowing rapid and cost-effective detection of hvKP, since no expensive equipment is needed. The RAA assay demonstrated greater sensitivity than the real-time PCR assay, detecting 20 copies/reaction of each gene.⁵³

A genome-wide association study (GWAS) approach identified known and novel accessory loci associated with the liver invasive phenotype.⁵⁴ Among the known biomarkes were *iroB* and *iucABD* loci, and among the novel biomarkers were *fepA* (a siderophore enterobactin receptor), *lutA* (a ferric aerobactin receptor) and *lucA/lucC* (siderophore biosynthesis proteins). Interestingly, *rmpA* was not associated with liver hvKP according to authors' statistical cut-off ($P < 10^{-10}$) which was explained due to possible frameshifted and truncated gene sequences, owing to the increased fitness cost of its maintenance and expression among CR-hvKP.⁵⁵

Another study investigated the identification of capsular serotypes of hypermucoviscous *K. pneumoniae*: K1, K2, K54 and K57, by combining Raman spectroscopy with one-dimensional convolutional neural networks (1-D CNN). Capsular serotypes were

classified with 96% accuracy using less than 30 Raman features after dimensionality reduction.⁵⁶

Among phenotypic methods, Wu et al. proposed the use of a certain concentration of potassium tellurite based on MacConkey medium to selectively culture *terW*⁺KP. The *terW* gene, associated to tellurium resistance, has been described together with *iutA-rmpA-silS* gene-derived locus and present in plasmids in CG23, CG65, and CG86. These bacterial groups commonly cause community-acquired purulent infections being closely related to specific hvKP strains.⁵⁷

Clinical manifestations, risk factors and treatment

Hypervirulent *K. pneumoniae* was first recognized causing a new invasive syndrome, liver abscess, however its association with other types of diseases is increasing. Liver abscess infection has been commonly associated with metastatic spread to distant sites: eye, lung, central nervous system (CNS), soft tissue and bones, and less commonly, abdominal and pelvic organs.⁴ The dissemination to multiple sites seems to be related to a higher capacity to invade tissue from bloodstream and to survive host defense factors.² The mechanisms by which this occurs is unclear but a high titer of hvKP during bacteraemia, increased capsule production and tissue damage, either because of bacterial or host factors (unregulated host response), could be contributing factors.² In addition, hvKP can cause primary extrahepatic diseases, such as bacteraemia, pneumonia, urinary tract infections, biliary tract infections, musculoskeletal and soft tissue infections, and peritoneal infections.²

Immunodeficiency and certain severe diseases such as diabetes and malignancy have been described as risk factors.^{2,4} Nevertheless most hvKP infections are community acquired and often occur in otherwise healthy hosts, being acquisition or colonization important factors that could lead to infections and thus, pathogen exposure from high incidence geographic regions is considered a risk factor.² Occult disruptions in the skin, bacterial factors, and previous oral ampicillin or amoxicillin treatment, or the use of proton pump inhibitors, which could lead to an increase of enteric pathogens, have been described as possible mechanisms enabling hvKP to cross mucosal and/or epithelia barriers. Nevertheless, yet-to-be-defined host genetic differences could also be contributing to an increased intestinal binding and/or translocation or this bacteria.²

Source control and antibiotic therapy are key factors for hvKP infections management. Source control typically includes percutaneous drainage and surgical intervention to reduce metastatic spread. Empiric antibiotic therapy should consider both

Table 2

Treatment possibilities for carbapenem resistant hvKP, either by the acquisition of a plasmid carrying carbapenemase (CR-hvKP) or by the acquisition of a virulence plasmid (hv-CRKP).^{4,59}

Agent	Class A	Class B	Class D	Possible combination with	Observations
Aztreonam	–	+	+	Ceftazidime–avibactam	Effectiveness compromised in strains producing ESBL genes capable of hydrolysing aztreonam
Fosfomycin	+	+	+		
Colistin, Polymyxin B	+/-	+/-	+/-		Constraints based on its nephrotoxicity and limited clinical effectiveness
Tigecycline	+/-	+/-	+/-		Inadequate clinical efficacy against urinary tract infections (limited tissue penetration and rapid dispersion after intravenous administration)
Ceftazidime–avibactam	+	–	+	Aztreonam Metronidazole (intra-abdominal infections)	Superiority over polymyxin antibiotics due to absence of toxicities
Meropenem–vaborbactam	+	–	–		
Imipenem–relebactam	+	–	–		
Cefiderocol	+	+	+		Exploits pathogen's iron uptake requirement at infection sites ("Trojan horse") Adjunctive therapy needed for anaerobic and gram-positive bacteria

Table 3

Novel therapeutic approaches for life threatening bacterial infections.^{2,58,59}

Therapy	Advantages	Disadvantages and/or challenges
Nanoparticles Metallic (Ag, Cu, Au, CeO ₂ , ZnO, etc.), carbonaceous (GO, graphene and carbon nanotubes, borides) and nanocomposites (i.e. La ₂ O ₃ /Ag-GO).	Combination of nanomaterials and antibiotics for spatiotemporal antibiotic delivery to infection sites. Facilitate targeted interactions primarily with the cell wall and membrane components (via oxidative stress, mechanical disruption and thermal impacts). No development of antibiotic resistance.	Limited ability to penetrate the cytoplasm. Resistance mechanisms includes mutations in the efflux system, suppression of the outer membrane porin family, horizontal gene transfer, flagellin production, remodeling the cell envelope. Nano-microbes interactions still poorly understood. Other challenges includes ambiguous toxicity and capture by macrophages.
Antimicrobial peptides	Interact with outer membrane structures and lipids for membrane disruption, and with intracellular components (DNA, RNA, proteins) for bacterial cell function disruption. Utilization of nanoformulations for delivery. Large identification of antimicrobial peptides based on advanced machine learning techniques.	Susceptibility to degradation within the physiological environment. Possible disruption of natural microbiota. Limited information regarding their safety profile. Expensive manufacturing and lack of commercial platforms for its production. Bacterial defensive mechanisms: production of capsules and alterations in lipopolysaccharides, sequestration and degradation.
Phage therapy	Superior selectivity in targeting bacteria without disrupting the natural microbiota. Potential application especially for persistent infections. Phage engineering tools for drug attachment to phage surfaces and release at specific locations.	Risk of selecting phage-resistant microbes. Complexities in pharmacodynamics and pharmacokinetics. Interactions between phages and hosts.
Immunotherapy	Vaccines targeting capsular polysaccharides and antibacterial monoclonal antibodies targeting surface-exposed antigens or secreted toxins. Examples of monoclonal antibodies directed against the K1 capsule and the O-antigen in hvKP.	Overcoming antigenic diversity of the surface polysaccharides. Availability of multiple capsule and O-antigen monoclonal antibodies and point-of-care tests for rapid identification of capsule and O-antigen type.

antimicrobial resistant patterns and the site of infection^{2,4,58,59} (Table 1). hvKP strains that are carbapenem resistant are the most concerning and the optimal treatment is still unknown. Therapeutic options are those considered for managing CRKP infections, combining traditional medications such as aminoglycosides, aztreonam, polymyxins, and tigecycline with other therapeutic agents, and β -lactam- β -lactamase inhibitor combinations such as ceftazidime/avibactam, meropenem/vaborbactam and imipenem/relebactam^{4,59} (Table 2). Treatment should be tai-

lored and deescalated based on in vitro susceptibility results, local epidemiology and carbapenemase type.

In recent years, innovative therapeutics approaches are emerging for life-threatening bacterial infections, such as nanodrug delivery systems that enable enhanced target drug delivery; phage therapy, implemented so far as compassionate use and in which clinical trials are needed to establish its effectiveness; immunotherapy, such as vaccines and antibacterial monoclonal antibodies^{2,58,59} (Table 3). In addition to effective antibiotic therapy and the

development of novel approaches, infection control strategies are a key factor in combating high-risk *K. pneumoniae* infections, this includes transmission prevention, which involves the need of point-of-care testing, host defence and protection of vulnerable population^{2,59} (Table 1).

Conclusions

The epidemiology of hypervirulent strains of *K. pneumoniae* has changed in recent years, with their geographical distribution extending beyond Asian Pacific Rim and associated clones becoming more diverse. The plasmid transmission of virulence genes facilitates the appearance of the hypervirulent phenotype in clones not previously considered as such.

The exchange of plasmids with a high genetic load of virulence, resistance, or both between cKP and hvKP determines the appearance of MDR and hypervirulent strains. Of particular concern is the acquisition of virulence plasmids by high-risk clones, such as ST11, ST512 and ST147.

Molecular-based detecting methods of hvKP rely mostly on the presence of specific virulence genes *rmpA*, *rmpA2*, *iroN*, *iucA*, or *pag344*, and hypervirulent K serotypes (K1/K2). Clinical management of hvKP infections should include proper diagnosis, antimicrobial resistant patterns, with special concerning of carbapenem-resistant strains, and the site of infection.

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Conflict of interest

None to declare.

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