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REVIEW

Virulence Factors and Carbapenem-Resistance Mechanisms in Hypervirulent *Klebsiella Pneumoniae*

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Abstract: Hypervirulent *Klebsiella pneumoniae* (hvKP) has emerged as a novel variant of *K. pneumoniae*, exhibiting distinct phenotypic and genotypic characteristics that confer increased virulence and pathogenicity. It is not only responsible for nosocomial infections but also community-acquired infections, including liver abscesses, endophthalmitis, and meningitis, leading to significant morbidity and mortality. HvKP has been reported all over the world, but it is mainly prevalent in Asia Pacific, especially China. Moreover, hvKP can acquire carbapenemase genes resulting in the emergence of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP), which possesses both high virulence and drug resistance capabilities. Consequently, CR-hvKP poses substantial challenges to infection control and presents serious threats to global public health. In this paper, we provide a comprehensive summary of the epidemiological characteristics, virulence factors, and mechanisms underlying carbapenem resistance in hvKP strains with the aim of offering valuable insights for practical prevention strategies as well as future research.

Keywords: hypervirulent *Klebsiella pneumoniae*, virulence factors, plasmid, drug resistance

Introduction

Klebsiella pneumoniae is a common gram-negative opportunistic pathogen, commonly found colonizing the environment, skin, mucous membranes and gut. It plays a significant role in nosocomial infections, primarily causing urinary tract infections, bloodstream infections, abdominal infections, and pneumonia.[1] In the 1980s, the first case of primary liver abscess caused by *K. pneumoniae* was reported in Taiwan.[2] Researchers found that the virulence of the isolate was much higher than that of classic *K. pneumoniae* (cKP), it was first described as hypervirulent *K. pneumoniae* (hvKP). Unlike cKP, hvKP not only causes nosocomial infection but also fatal infections in healthy individuals via liver abscess, endophthalmitis and meningitis, which are often metastatic and disseminated, with significant morbidity and lethality.[3,4] The clinical characteristics of hvKP and cKP are shown in Table 1. HvKP strains can produce thick capsules, resulting in a hypermucoviscous phenotype, which can be judged by a string test (string ≥5 mm). Some researchers identify all *K. pneumoniae* with hypermucoviscous phenotype as hvKP.[5,6] However, the correlation between the hypermucoviscous phenotype *K. pneumoniae* and hvKP varies, and not all hvKP strains have the hypermucoviscous phenotype.[7–9] Harada et al[10] recorded that the accuracy of hvKP judged by a string test was 90%. Yu et al[11] used the combination of "(*rmpA / rmpA2*) + *iutA*" genes to judge hvKP. Russo et al[12] found that using the combination of five genes, *prmpA*, *prmpA2*, *peg-344*, *iroB* and *iucA*, to identify hvKP had a 95% accuracy. The *peg-344* and *iucA* genes further improved diagnostic efficacy. The *peg-344* gene encodes the inner membrane transporter located on the virulence plasmid. The sensitivity and specificity of *peg-344* for rapid screening for hvKP have been observed as 100% and 95%, respectively.[13] Currently, no uniform standard exists to define hvKP, which makes it difficult to compare data from different studies. Therefore, agreement on genetic markers to identify hvKP has become an urgent need. However, identifying hvKP, the string test lacks sensitivity and specificity, and it is more appropriate to utilize multiple genes rather than a single one. The

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Table 1 The Clinical Characteristics of hvKP and cKP

Characteristics	Hypervirulent *Klebsiella pneumoniae* (hvKP)	Classic *Klebsiella pneumoniae* (cKP)
Infection type	Community/nosocomial, community predominant	Nosocomial
Host	Healthy individuals	Immunocompromised patients
Infection sites	Pyogenic liver abscess, endophthalmitis, meningitis, necrotizing fasciitis	Urinary tract infections, bloodstream infections, abdominal infections, pneumonia
Metastatic	Common	Uncommon
Prevalent region	Asia Pacific, sporadically in Africa	Worldwide
Phenotype	Hypermucoviscosity, string≥5mm	Non-hypermucoviscosity, string<5mm
Serotypes	K1, K2, K5, K20, K54, K57	K1-K78

combination of "(*rmpA/rmpA2*) + *peg-344* + *iro* + *iuc*" serves as a more accurate molecular marker for hvKP at present. Exploring novel detection techniques and methods will contribute to enhanced accuracy in identifying hvKP.

Historically, most hvKP strains did not express a phenotype of high drug resistance and high virulence and were generally thought to remain highly sensitive to most antibiotics.[14] In recent years, however, with the widespread application of broad-spectrum antibiotics, increasing numbers of multi-drug-resistant hvKP, especially carbapenem-resistant hvKP (CR-hvKP) have been reported worldwide.[15,16] The emergence of CR-hvKP heralds a potential new global public health disaster.[17] HvKP strains typically contain a virulence plasmid with a variety of virulence encoding genes, and the plasmid has rapidly adapted to coexist with different strains of *K. pneumoniae* through constant genetic changes during transmission.[18] In addition, the genome of *K. pneumoniae* can acquire resistance genes through plasmids and mobile genetic elements (MGE), leading to the emergence of multi-drug resistant (MDR) and extremely drug-resistant (XDR) strains.[19] Data from China's antimicrobial resistance surveillance system showed that the resistance rate of *K. pneumoniae* to imipenem and meropenem was 3.0% and 2.9%, respectively in 2005, and increased to 23.1% and 24.4%, respectively in 2021.[20] The emergence of CR-hvKP has been documented globally. In 2014, Cejas et al from Argentina reported the first South American isolate of CR-hvKP producing KPC-2.[21] Subsequently, in India, 3 CR-hvKP isolates carrying OXA-232, OXA-181, and OXA-1 had their complete genome sequences reported in 2016.[22] Additionally, Arena et al identified a CR-hvKP isolate from a patient with a liver abscess in Italy during 2017.[23] Furthermore, in 2021, a hospital in Egypt isolated a CR-hvKP isolate harboring both *blaKPC-2* and *blaNDM-1*.[24] Notably, reports of CR-hvKP have also emerged from various regions within China including Beijing, Shanghai, Zhejiang, Heilongjiang, Shandong and Henan.[25–27] The increase of CR-hvKP has brought great challenges to clinical anti-infection treatment and nosocomial infection prevention and control. To understand the characteristics of hvKP more comprehensively, we review the prevalence, virulence factors and drug resistance mechanisms of hvKP.

Epidemiology

In the 1980s, seven cases of hvKP were first reported in Taiwan area. In addition to liver abscesses, these patients suffered from concurrent pyogenic meningitis and endophthalmitis. Despite active antibacterial treatment, six of them eventually became blind and one was visually impaired.[2] Subsequently, hvKP was found in many regions of Asia[28,29] as well as other countries in Europe,[30] America[21,31] and Africa.[24] A multi-center study conducted by Peking University revealed that out of 230 *K. pneumoniae* isolates from 10 cities in China, 37.8% were hvKP, with the highest rate of 73.9% in Wuhan.[32] Another study showed that hvKP accounted for up to 90.9% of pathogens causing pyogenic liver abscesses.[33] A Korean study indicated that the hypermucoviscous phenotype was present in approximately 42.2% of *K. pneumoniae* causing bloodstream infections.[34] However, hvKP was reported sporadically in some countries in Europe, America and Africa, and the rates were generally below 10%.[35–37] Overall, hvKP was mainly prevalent in the region of Asia Pacific, and there was a certain proportion of hvKP colonized in Asian populations, which may be related to differences in genetic susceptibility or differential distribution.

HvKP clonal group 23 (CG23) has its unique lineage. In Taiwan, Singapore and mainland China, 37%~64% of hvKP isolates belong to CG23, mainly including ST23, ST26, ST57 and ST163.[38] Among these types, ST23 is the most common and correlate with K1 serotype. A study in China found that 96.2% of ST23 hvKP isolates belonged to K1 serotype and were closely associated with the formation of liver abscess.[39] A meta-analysis[40] showed that 394 hvKP isolates were grouped into 50 different sequence types (STs); however, the most common were ST23, ST11, ST65 and ST86. Of these, 113 isolates belonged to K1 serotype, while 86 were isolated from patients with liver abscess, indicating that ST23-K1 was closely related to liver abscess. The STs of K2 serotype isolates exhibited a range of diversity, including ST65, ST66, ST86, ST373, ST374, ST375, ST380 and ST434; of which ST65 and ST86 predominated, associating with invasive infection.[41,42] CR-hvKP has emerged with different STs from hvKP. The first isolate of ST23-CR-hvKP producing *blaKPC-2* carbapenemase gene was reported in 2014 in South America.[21] However, subsequent nosocomial outbreaks were primarily characterized by the emergence of the novel clonal ST11-CR-hvKP in Zhejiang, China, in 2018.[43] In 2020, researchers from Singapore studied 556 CRKP isolates collected from 6 public hospitals over 6 years, of which 18 CR-hvKP isolates producing *blaKPC-2* gene were mainly ST23-K1 and ST65-K2.[44] The STs of CR-hvKP differed regionally; ST11, ST23 and ST258 dominated in the USA, India, Russia, Egypt and Italy, while in China, 80% of CR-hvKP isolates belonged to ST11 producing *blaKPC-2*, along with a small number of ST23, ST268, ST65 and ST692.[16,45,46] Thus, ST23 was the main type of low-drug resistant hvKP strain in China, whereas ST11 dominated CR-hvKP strains.

Virulence Factors

Currently, the identified virulence factors of hvKP include capsular polysaccharide, siderophore, virulence genes, virulence plasmids, lipopolysaccharide, and fimbriae. Of these, capsular polysaccharide and siderophore are considered the most crucial contributors to pathogenesis.

Capsular Polysaccharide

Capsular polysaccharide (CPS) is the extracellular polysaccharide matrix surrounding bacteria, and an important pathogenic substance of hvKP. It not only prevents the phagocytosis of bacteria by immune cells, but it also hinders the bactericidal effect of antimicrobial peptides by binding to the molecules at the end of the outer membrane, playing an important role in the process of bacterial adhesion and anti-phagocytosis.[47] Based on the different capsular antigens of hvKP, *K. pneumoniae* can be divided into at least 78 subtypes, including K1, K2, K5, K20, K54 and K57, which are closely related to invasive infection, among which K1 and K2 are the most common serotypes.[48] In a study from Zhejiang, China, K1 and K2 accounted for 23.8% and 42.9%, respectively, of the hvKP isolates causing invasive infections.[42] A Korean study showed that hvKP isolated from the urine of hospitalized patients were more common in K1 and K2 types than cKP.[49] Studies in the USA, Canada and the UK also showed that hvKP isolates were dominant in K1 and K2 serotypes.[36,50,51] K1 and K2 serotypes are associated with aggressive disease and enhanced pathogenicity in peritonitis mouse models,[52] possibly due to the lack of mannose or rhamnose recognized by macrophage lectin receptors, which can then escape the host immune response, creating a favorable environment for hvKP survival and reproduction.[32] The serotypes K1 and K2 serve as the fundamental pathogenic factors of hvKP, playing a crucial role in its heightened virulence.

Siderophore

Iron is a crucial nutrient element for the growth and reproduction of bacteria. However, its direct utilization is hindered by the low solubility of Fe^{3+}. Consequently, bacteria absorb iron from the host by secreting siderophore to provide energy, thereby enhancing their virulence and accelerating the infection process. Thus, siderophore is an important virulence factor for bacteria. The siderophore activity of hvKP is 6–10 times higher than that of cKP.[53] Common siderophores include aerobactin, yersiniabactin, salmochelin and enterobactin, which are encoded by the genes *iuc, ybt, iro* and *ent*, respectively. After knocking out these genes, the amount of siderophore declined to various degrees, leading to reduced virulence and knockout of the gene encoding aerobactin reduced virulence significantly more than the other gene knockout isolates.[53] Aerobactin, a hydroxamic acid-based siderophore that accounts for more than 90% of the total siderophore activity, is encoded by the *iucABCD* operon, and its membrane protein receptor is encoded by the *iutA* gene.[54] When hvKP lacking the *iucA* gene was cultured in vitro with ascites, urine, or serum, siderophore production

decreased by 95%, 94% and 100%, respectively, compared to the wild-type strains.[53] Disrupting the synthesis of aerobactin could thus reduce the growth or viability of hvKP in human ascites and serum, while disruption of enterobactin, yersiniabactin, and salmochelin synthesis cannot achieve the same effect, indicating that aerobactin is a key virulence factor in hvKP.

Virulence Genes

The hypermucoviscous phenotype is a crucial characteristic of hvKP, primarily attributed to the presence of capsule. Capsule synthesis is predominantly regulated by the regulator of mucoid phenotype (rmp) operon that comprises *rmpA, rmpD* and *rmpC* gene, in which the *rmpA* gene autoregulates the operon, *rmpD* confers the hypermucoviscous phenotype, and *rmpC* promotes CPS biosynthesis.[55,56] In 1989, *rmpA* was first reported to be associated with hypermucoviscous phenotype and hypervirulence of hvKP,[57] which consisted chromosome-mediated *c-rmpA* and plasmid-mediated *p-rmpA* and *p-rmpA2*.[58] Studies indicate that *rmpA* is related to the hypermucoviscous phenotype of hvKP strains. After knocking down the *rmpA* gene on the plasmid, the viscosity of the isolate was reduced, and virulence concomitantly decreased.[59] A study in Singapore showed that hvKP isolated from patients with community-acquired liver abscesses all carried the *rmpA* gene.[4] Most hypermucoviscous isolates carry the *rmpA* gene, although a small number of isolates do not carry it,[60,61] suggesting that the hypermucoviscous phenotype of hvKP isolates may not be completely mediated by the *rmpA* gene, and other regulatory mechanisms may exist. Some isolates with the non-hypermucoviscous phenotype were found to carry the *rmpA* gene,[62] which may be attributed to *rmpA* mutation that reduced transcription of *rmpD* and *rmpC*, resulting in the absence of hypermucoviscous phenotype. *RmpD* is an essential determinant for hyperviscosity phenotype, which is located between *rmpA* and *rmpC*, within an operon regulated by *rmpA*. *RmpC* is another regulator of capsule gene expression, which is encoded downstream of *rmpA*. Expression of *RmpD* is sufficient to confer hypermucoviscous phenotype on *rmpA* mutants, while overexpression of *rmpC* elevates cps expression even in the Δ*rmpA* isolates.[56]

Mucoviscosity-associated gene A (*magA*) was discovered and reported by scholars from Taiwan in 2004.[63] It is located on the chromosome and encodes outer membrane protein involved in the production of exopolysaccharide. *MagA* is considered as the allele of K1 serotype of polymerase gene wzy in cps gene cluster, thus named wzy_K1. It is responsible for regulating the synthesis of capsular polysaccharides; however, this regulation is limited to K1 isolates.[64]

Virulence-Associated Plasmids

HvKP typically carries two similar large plasmids: pK2044 (224 kbp) and pLVPK (219 kbp), both of which belong to IncHI1/IncFIB plasmids. The pLVPK plasmid carries the gene *rmpA/rmpA2*, the aerobactin-encoding gene *iuc* and the salmochelin-encoding gene *iro*, which are known as virulence plasmid-associated genes.[1,3] Strain virulence is reduced significantly when the plasmid pLVPK is lost. Plasmids containing different sizes of *iuc, rmpA*, and *rmpA2* are referred to as pLVPK-like plasmids, and these plasmids also play an important role in mediating the high virulence of hvKP. Ye et al[33] and Struve et al[65] found that hvKP isolated from patients with liver abscesses or community-acquired pneumonia all carried pLVPK-like plasmids that contained *iuc, iro, rmpA* and *rmpA2*. Another study showed that a high-resistance CRKP isolate that acquired a 178 kb pLVPK-like plasmid from an hvKP isolate, evolved into a CR-hvKP strain.[43] Thus, some studies have proposed the combination of virulence plasmid genes *rmpA* + *rmpA2* + *iuc* or the pLVPK plasmid as biological markers of hvKP strains.

Mechanisms of Carbapenem Resistance

Carbapenems have long been considered the last line of defense for the treatment of MDR gram-negative bacteria. However, in recent years, the widespread application of broad-spectrum antibiotics has accelerated the emergence of carbapenem-resistant Enterobacteriaceae, especially *K. pneumoniae*. Carbapenem resistance in *K. pneumoniae* primarily arises from the production of carbapenemase, a trait that is widely disseminated through plasmids, integron conjugators (ICE), integrons, transposons, and other movable genetic elements. These movable genetic elements facilitate transfer between *K. pneumoniae* strains, leading to an elevated resistance rate of hvKP to carbapenems. Research indicates that, to date, CR-hvKP has evolved mainly through the following three modes.

HvKP Acquiring Carbapenem-Resistant Plasmids

Drug-resistant plasmids play a crucial role in the transmission of MDR bacteria, and *K. pneumoniae* exhibits a robust capacity to incorporate mobile elements, which is the primary factor contributing to its high level of drug resistance. Studies showed that hvKP strains can acquire drug resistance by trapping resistance plasmids such as IncFII, IncN, and IncL/M.[62,66] ST23 and ST65, the most prevalent clones of hvKP, became resistant to carbapenems by capturing the plasmid carrying the *blaKPC-2* gene.[67,68] In 2018, Dong et al[69] from the Hong Kong Polytechnic University obtained whole genome sequences of three CR-hvKP isolates using Oxford Nanopore sequencing technology, and confirmed the unique genetic characteristics of the plasmids carried by the isolates. Homologous regions were observed between the unbound virulence plasmid and the drug-resistant plasmid bound to *blaKPC-2*, suggesting that the transmission of virulence plasmids from ST23 hvKP to ST11 CRKP may be mediated by the co-integrated transfer of these two plasmids. In addition, studies in vitro showed that K2-ST65 hvKP isolates obtained the resistance plasmid carrying the *blaKPC-2* gene from ST258 CRKP isolates by conjugation.[70] This research indicates that the drug-resistant plasmids carrying *blaKPC-2* genes can transfer between hvKP clones, thus forming CR-hvKP strains. In 2017, researchers in Jiangxi, China discovered an hvKP isolate carrying both *blaKPC-2* and *blaNDM-1* carbapenemase genes for the first time. Whole genome sequencing (WGS) and virulence phenotype tests showed that the CR-hvKP isolate retained high virulence characteristics after acquiring the two carbapenemase genes.[71] In recent years, CR-hvKP isolates producing NDM and OXA enzymes have appeared in India. K2-ST86 isolates producing *blaKPC-2* and K1-ST23 isolates producing *blaKPC-2* and *blaNDM* have also been reported in Canada, USA and the UK.[50,68,72] Thus, the acquisition of a carbapenemase plasmid may promote the rapid adaptation to CR-hvKP, which will eventually lead to worldwide abundance.

CRKP Acquiring Hypervirulent Plasmids

The pLVPK-like virulence plasmid is an important factor responsible for the high virulence of *K. pneumoniae*.[73] Traditionally, it was thought that cKP in ST11, ST258 and other major prevalent clones had significantly weaker pathogenicity than hvKP due to the lack of a pLVPK-like virulence plasmid.[74] However, Yao et al[25] reported on a fatal infection outbreak caused by ST11 cKP strains and found them to be CR-hvKP strains carrying a pLVPK-like virulence plasmid. Since then, CR-hvKP strains carrying pLVPK-like virulence plasmids have been reported successively in Beijing, Shenzhen, Hangzhou, Zhengzhou and other places in China,[25,75–77] indicating that cKP can evolve into CR-hvKP by acquiring a pLVPK-like virulence plasmid. A recent study found that pLVPK-like virulence plasmids can transfer from hvKP strains to ST11-CRKP strains by the self-transferring IncF plasmid.[78] Additionally, Xie et al[79] discovered that high virulence coding genes can be extensively transmitted to MDR *K. pneumoniae* through various plasmid-mediated conjugation mechanisms. Subsequently, the same team investigated the molecular mechanism of acquiring virulence plasmids by ST11 CR-hvKP strains, finding it involved homologous recombination and insertion of IS26 and IS903B sequences. A fusion plasmid formed between an Incl1 conjugation plasmid and a small Col RNAI plasmid promoted different ST-type *K. pneumonia*e to combine, particularly ST11 and ST258 *K. pneumoniae*, creating a CR-hvKP.[80] The discovery that fusion events lead to the generation of novel virulence factors by *K. pneumoniae* and enhance the transmission of these virulence factors offers new insights into the mechanism underlying plasmid-mediated virulence dissemination in *K. pneumoniae*.

K. Pneumoniae Acquiring Both Hypervirulence and Carbapenem Resistance Hybrid Plasmids

Plasmids are extra-chromosomal genetic material that play an important role in the transmission of genetic elements related to drug resistance and virulence. The evolution and genetic diversity of multiple drug resistance and virulence plasmids have led to the emergence of hvKP, which has both high virulence and high drug resistance. Plasmids in *K. pneumoniae* contain virulence genes and drug resistance genes encoding specific functions and exploit multiple mechanisms to spread among bacteria along with other genetic factors such as integrons and transposons, allowing bacteria to survive in hostile environments.[81] In 2018, researchers in Hong Kong, China found a plasmid carrying both the virulence gene *rmpA2* and the carbapenemase gene *blaKPC-2* from a clinically isolated hvKP isolate and named it pKP70-2. The plasmid was formed by IS26 mediating the insertion of *blaKPC-2* and the dfrA resistance mobile element into the virulence plasmid.[82] In the same year, a hybrid virulence plasmid pVir was discovered in the isolate TVGHCRE225 using WGS in Taiwan. The first

region of the plasmid had 99% homology with plasmids pK2044 and pLVPK, containing virulence genes *rmpA* and *rmpA2*, and the second region was highly similar to the pPMK1-NDM hybrid plasmid.[83] These strains can thus express both high virulence and high resistance phenotypes, which indicates that high virulence genes and high resistance genes can coexist on a single plasmid for transmission, contributing to the widespread prevalence of CR-hvKP strains.

Conclusion

The high virulence and lethality of hvKP have attracted widespread concern around the world. The high virulence of hvKP is regulated by a variety of factors, such as capsular polysaccharide, siderophore, virulence genes, lipopolysaccharide, and fimbriae. Acting both alone and together, these virulence factors can lead to the hypervirulence features of hvKP. Thus, virulence factors may serve as potential targets for the development of hvKP vaccines and drugs, offering new ways and insights. With the global dissemination of CR-hvKP, effective antimicrobial drugs play a crucial role in control of CR-hvKP infections. Additionally, investigating the transmission and transfer mechanisms of its high virulence genes and drug resistance genes could prove effective means to develop new therapeutic drugs and impede its spread, thus becoming a pivotal focus of future research. At present, the key to preventing the transmission of CR-hvKP is to control the infection comprehensively by strengthening active surveillance and developing new methods to curb its spread.

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Disclosure

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