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Review

"Superbugs" with hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*: the rise of such emerging nosocomial pathogens in China

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ABSTRACT

Although hypervirulent *Klebsiella pneumoniae* (hvKP) can produce community-acquired infections that are fatal in young and adult hosts, such as pyogenic liver abscess, endophthalmitis, and meningitis, it has historically been susceptible to antibiotics. Carbapenem-resistant *K. pneumoniae* (CRKP) is usually associated with urinary tract infections acquired in hospitals, pneumonia, septicemias, and soft tissue infections. Outbreaks and quick spread of CRKP in hospitals have become a major challenge in public health due to the lack of effective antibacterial treatments. In the early stages of *K. pneumoniae* development, HvKP and CRKP first appear as distinct routes. However, the lines dividing the two pathotypes are vanishing currently, and the advent of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) is devastating as it is simultaneously multidrug-resistant, hypervirulent, and highly transmissible. Most CR-hvKP cases have been reported in Asian clinical settings, particularly in China. Typically, CR-hvKP develops when hvKP or CRKP acquires plasmids that carry either the carbapenem-resistance gene or the virulence gene. Alternatively, classic *K. pneumoniae* (cKP) may acquire a hybrid plasmid carrying both genes. In this review, we provide an overview of the key antimicrobial resistance mechanisms, virulence factors, clinical presentations, and outcomes associated with CR-hvKP infection. Additionally, we discuss the possible evolutionary processes and prevalence of CR-hvKP in China. Given the wide occurrence of CR-hvKP, continued surveillance and control measures of such organisms should be assigned a higher priority.

1. Introduction

Klebsiella pneumoniae is an opportunistic bacterium that can cause serious organ damage and life-threatening diseases. Based on its phenotypic and genotypic features, *K. pneumoniae* can be categorized into two types: classic *K. pneumoniae* (cKP) and hypervirulent *K. pneumoniae* (hvKP), each of which presents unique challenges for clinicians [1,2]. CKP commonly causes infections in patients who are immunocompromised, have concomitant conditions, or have a preexisting barrier breakdown (such as intravascular devices, endotracheal tubes, and surgical incisions) [3,4]. This pathotype is able to pick up several components that confer antibiotic resistance. The US Centers for Disease Control and Prevention reported the occurrence of carbapenem-resistant *Enterobacteriaceae* strains, which frequently cause untreatable or difficult-to-cure infections in hospital patients because of limited treatment options [5]. In China, carbapenem-resistant *K. pneumoniae* (CRKP) strains account for about 90% of clinical carbapenem-resistant *Enterobacteriaceae* (CRE) infections [5]. This high prevalence of CRKP strains in the healthcare system is expected as widespread antimicrobial use gives them a selective advantage. The characteristics of hvKP are different from those of cKP. Additionally, most reported cases of hvKp infections were contracted in the community. Characteristics highly suggestive of hvKp infection include the ability to infect healthy people of any age and the tendency of infected patients to present with multiple infection sites and/or develop subsequent metastatic spread (such as pyogenic liver abscess, endophthalmitis, and meningitis) and an unusual occurrence for cKP [2,6]. Although patients with hvKP infections often

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experience worse symptoms than those with cKP because the illness progresses more quickly, the initial isolates of hvKp are sensitive to antimicrobials, and the condition can be controlled quickly with the use of antibiotics [6]. Recently, clinicians have encountered an even bigger challenge due to the interaction between the virulence factors present in hvKP and the antibiotic resistance determinants present in cKP. More and more *K. pneumoniae* strains with both characteristics have been isolated recently; these strains cause the development of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) and devastating clinical outcomes [7]. CR-hvKP is a newly reported nosocomial pathogen, first and mainly described in China. This nosocomial pathogen demonstrates characteristics of both carbapenem resistance and hypervirulence and causes serious diseases in hosts and hospital outbreaks [8]. The convergence of hypervirulence and carbapenem resistance is the result of multiple mechanisms. These mechanisms include the horizontal transfer of carbapenem-resistant genes from the CRKP to the hvKP, the acquisition of a hypervirulence plasmid carrying virulence-encoding genes by the CRKP, and the development of CR-hvKP from *K. pneumoniae* through the uptake of a hybrid plasmid containing both hypervirulence and carbapenem-resistance genes.

The objective of this review was to summarize our current knowledge about this threatening and evolving pathogen. To achieve this, we explored recent progress in research on CR-hvKP, focusing on aspects related to the mechanisms underlying hypervirulence and carbapenem resistance in *K. pneumoniae*, the molecular basis for its clinical identification, the clinical features of CR-hvKP infection, as well as the epidemiology of CR-hvKP in China. Additionally, to better understand the traits of CR-hvKP, we analyzed its evolutionary processes of resistance and virulence.

2. Virulence factors of CR-hvKP

In this section, we discuss factors responsible for the virulence of CR-hvKP and provide an overview of the various methods used to distinguish between cKP strains and hvKP strains. These methods include capsule and hypermucoviscosity, siderophore, virulence plasmids and ICEKp, other virulence-associated genes, and clinical manifestations, which are presented in Fig. 1.

2.1. Capsule and hypermucoviscosity

The primary factors responsible for the hypermucoviscosity (HMV) phenotype in *K. pneumoniae* are the polysaccharides in the outermost capsule, which contribute to its virulence [2,9]. However, the thick hypercapsule can act as a physical obstacle, consequently hindering the absorption of DNA and horizontal transfer of genes, which partly explains why hvKP are less prone than cKP to harboring antimicrobial resistant genes [10].

At least 79 capsular serotypes have been identified based on various structures and antigens and various polysaccharide components of the capsule [11]. The K-types occurring in hvKP isolates are fewer than those in cKP strains, with K1, K2, K5, K16, K20, K54, K57, and KN1 being the most prevalent K-types in hvKP [12,13]. K1 and K2 are the most common capsular serotypes and the major pathogenic capsular serotypes that can cause invasive infectious diseases [14]. The K1and K2 hvKP was significantly more resistant to both intracellular and extracellular killing of neutrophils than cKP isolates [15]. The serotype is, therefore, a potential virulence factor, and the capsular serotypes K1 and K2 are representative of hvKP in general [16]. Recently, a bioconjugate vaccine against the K1/K2 serotype was developed to treat hvKP infections [17]. When CRKP began gaining the hvKP virulence plasmid, hvKP strains are thought to have increased the variety of capsule types (e.g., KL47 and KL64), which previously only appeared in cKP [18].

Genes responsible for capsule production are located on the chromosome's capsular polysaccharides synthesis (CPS) region. Multiple factors in this region can regulate capsule production, including transcriptional antiterminator (*rfaH*) and a number of proteins involved in capsule biosynthesis, such as Wzi, Wza, Wzc, WcaJ, and WbaP [19,20]. Additionally, high capsule productivity is also associated with various virulence genes, such as A and B genes (*rcsA* and *rcsB*) that regulate capsule synthesis, *Klebsiella* virulence regulators (*kvrA* and *kvrB*), and the regulator of mucoid phenotype A and A2 (*rmpA* and *rmpA2*) [21–23]. Previously, it was believed that HMV and CPS overproduction had similar characteristics, resulting in the incorrect linking of the *rmpA* locus with the HMV phenotype [24]. Notably, not all *rmpA/rmpA2* genes can increase the expression of the capsule, and strains carrying the *rmpA*/*rmpA2* genes but lacking a hypercapsule phenotype are also identified [25]. Walker et al. [26] revealed that *rmpA* stimulates the activation of a transcriptional regulator that controls the expression of CPS genes, including *rmpD* and *rmpC*. Inactivating *rmpA* causes loss of the HMV phenotype as a result of reduced expression of *rmpD*, while a decrease in capsule synthesis is attributed to reduced expression of *rmpC*. Additionally, the absence of *rmpC* lowers CPS production without affecting the HMV phenotype [27], whereas *rmpD*, which encodes a small membrane protein causing HMV phenotype, has no impact on capsule production [26], demonstrating that the development of HMV and CPS are independent features. Although *rmpD* is functional, strains with mutations in the CPS biosynthesis genes (*wcaJ* and *manC*) also lose HMV, indicating that some components of CPS production are required for HMV [26]. RmpD can alter Wzc-mediated CPS synthesis in larger polysaccharide chains of a more consistent length, which is a crucial component of the HMV phenotype of hvKP [28]. Nucci et al. [29] revealed that through mutations in *wzc,* hvKP and MDR could easily evolve HMV. Recent studies have shown that when obtaining the plasmid carrying bla_{KPC-2}, "CR-hvKP" strains quickly adapt to minimize the energy load by reducing CPS production and virulence through the mutation of *rfaH* and *wcaJ* in K1 and K2 CR-hvKP, respectively [30]. Mutations in *wcaJ* have been found in four ST23-K1 CR-hvKP, in which they caused little capsule synthesis, virulence decline, low fitness cost, and high conjugation frequency of the bla_{KPC-2} plasmid, highlighting that reduced CPS is a potential factor facilitating hypervirulence and carbapenem resistance [9]. Mutations in *wzc* occur in ST11-KL64 CR-hvKP strains, causing HMV phenotype, which increases virulence and resistance to macrophage phagocytosis and has a negative effect on bacterial fitness. However, mutations in *wcaJ* result in a non-mucoid phenotype with reduced virulence and resistance against macrophage phagocytosis, and CPS has a high impact on mucoid phenotypic alternations [31]. Furthermore, overexpression of *rmpA* in *rmpA*-low-expression cKP isolates can enhance virulence in a mouse infection experiment [32]. All the studies conducted so far demonstrate that in CR-hvKP strains, CPS can be used as a substitute virulence marker.

Regulation of most of the aforementioned genes is directed at the CPS cluster, which may form a complex network to impact capsule synthesis. However, the hypercapsule is not the sole cause of HMV, and both may serve distinct functions during pathogenesis [29,33]. Not all hvKP strains exhibit HMV, and some cKP strains also display an HMV phenotype [34]. Further research is needed to better understand the differences between hypercapsule and HMV in hvKP, which could have implications for the identification and treatment of hvKP strains.

2.2. Siderophore

A crucial component needed for vital metabolic activities is the metal iron in bacteria. As the free Fe^{3+} is insoluble under physiolog-

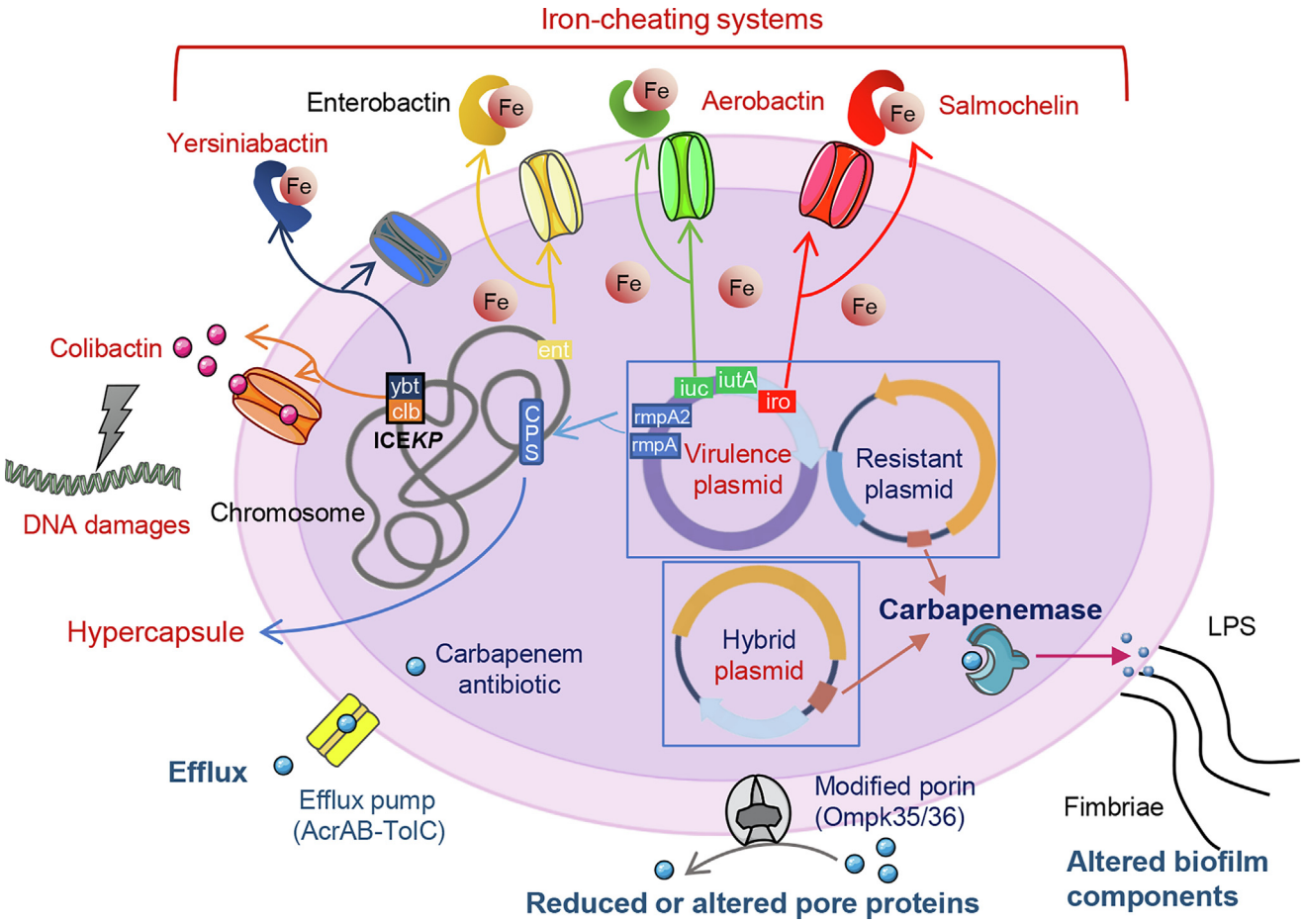

Fig. 1. Virulence features and carbapenem-resistance mechanisms of CR-hvKP. Virulence features (in red) of CR-hvKP include both chromosomal and plasmid-encoded features that increase virulence. Additional factors that contribute to the pathogenesis of all *Klebsiella pneumoniae* (in black) include ent, fimbriae, and LPS on the surface. The carbapenem-resistance mechanisms of CR-hvKP are shown in blue. The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license (creativecommons.org/licenses/by/3.0/).

ical conditions, human hosts normally require a siderophore-dependent iron absorption system for iron ingestion. Similarly, most bacteria, including *K. pneumoniae*, have this system that absorbs iron more effectively than the host; it is a common method of collecting iron to survive the host competition [16]. Small molecules known as siderophores are produced by bacteria inside the cell and exported to bind iron in the environment with an incredibly high affinity before being brought back into the cell to supply the bacterium with iron needed for growth.

hvKP isolates can produce more siderophores than cKP isolates [35]. The hvKP isolates express four siderophores, including enterobactin, yersiniabactin, salmochelin, and aerobactin, among which yersiniabactin, salmochelin, and aerobactin are more common in hvKP than in cKP [36]. The genes required for enterobactin and yersiniabactin biosynthesis are located on the chromosome in the *ent* cluster and *ybt* loci, respectively, while the biosynthesis of salmochelin and aerobactin occurs in *iroBCDN* and *iucABCDiutA* clusters of virulence plasmids, respectively [37]. In hvKP isolates, aerobactin contributes primarily to hypervirulence under experimental conditions, constituting about 90% of the total siderophore synthesis. Additionally, after pneumonic and subcutaneous infection, mice infected with hvKP mutants with aerobactin deficiency displayed lower susceptibility than mice infected with hvKP isolates deficient in enterobactin, salmochelin, or yersiniabactin [35].

Furthermore, a new iron transport gene, *kfu*, was discovered by studying the genomic sequence of the hvKP NTUH-K2044 strain; this gene is common in hvKP strains and is also regarded as a potential virulence factor [3]. The various iron transport systems in *K. pneumoniae* are complementary as they can play a role under different microenvironmental conditions during infection.

The regulatory mechanism of siderophores in hvKP involves a complex interplay of iron availability, iron-responsive regulators, and two-component systems. Firstly, hvKP strains sense the environmental iron concentration and alter their production of siderophores to change their ability to obtain iron from the host, which is mostly accomplished through iron-responsive regulators. These regulators sense the iron concentration and modulate the expression of genes involved in siderophore synthesis and transport. For example, the ferric uptake regulator (Fur) acts as a repressor of siderophore production under iron-rich conditions, while the demetallating Fur allows efficient acquisition of iron and enhances the fitness of the pathogen under iron-poor conditions [38]. Furthermore, hvKP strains also use two-component systems (QseBC and CpxAR) to regulate siderophore production and the expression of other virulence genes involved in the development of biofilms, the bacterial type VI secretion system, and type 1/3 fimbriae [39,40]. More interestingly, the CpxAR system can also negatively regulate the expression of type 3 fimbriae by modulating cellular iron levels, further demonstrating the complex interactions among virulence factors [39]. Overall, these mechanisms ensure that hvKP strains can efficiently scavenge iron from the host environment and survive in iron-limited conditions.

2.3. Virulence plasmids and ICEKp

To date, research on the 224-kb virulence pLVPK from strain CG43 (K2, ST86) and plasmid pK2044 from strain NTUH-K2044 (K1, ST23) has been extensive [41,42]. *K. pneumoniae* virulence plasmids often carry multiple virulence factors, including HMV phenotype regulatory genes (*rmpA*/*rmpA2*), siderophore-related gene clusters *(iucABCDiutA*, *iroBCDN*, *ybtAEPQTUX,* and *entABC-DEFS*), as well as the genes for tellurite and silver resistance (*ter-ABCDEWXZ* and *silCERS*). Among them, the most precise and distinctive molecular markers on virulence plasmids for identifying hvKP are *iroB*, *peg-344*, *iucA*, *rmpA,* and *rmpA2* [43]. CRKP strains have acquired virulence plasmids and can cause devastating clinical outcomes [8,44]. For example, after acquiring a pK2044 virulence plasmid, an ST11-CRKP (JS187) developed a dense capsule, exhibiting carbapenem resistance and hypervirulence [30].

Apart from virulence plasmids, a type of mobile genetic element (MGE) called ICEKp can also spread these virulence factors throughout bacterial communities through horizontal gene transfer in *K. pneumoniae*. Fourteen types of ICEKp have been reported in hvKP (ICEKp1-ICEKp14), and the yersinia high-pathogenicity island, on which the *ybt* locus gene is located, is a significant feature of most ICEKp [45]. Apart from ICEKp1 of NTUH-K2044, ICEKp10, which is similar to KPHPI208 genomic island in liver abscess strain 1084, appears to be the most prevalent ICE in CG23 hvKP [46]. The ICEKp pathogenicity island of most CC23 isolates contains the genes for yersiniabactin, colibactin, and microcin E492 [46]. A polyketide-peptide genotoxin called colibactin causes typical symptoms of bacterial meningitis and severe DNA damage in eukaryotic cells [47].

2.4. Other virulence factors

Klebsiella pneumoniae has type 1/3 fimbriae [48]. The hvKP NTUH-2044 genome has seven new fimbrial gene clusters, namely, *kpa, kpb, kpc, kpd, kpe, kpf*, and *kfg*, with *kpc* being strongly related to K1 serotype hvKP [49]. The small protein KP1_4563 in NTUH-K2044, which contains the DUF1471 domain, inhibits the activity of type 3 fimbriae [50]. Additionally, although serotype K1 hypermucoviscous isolates have genes for type 1/3 fimbriae, they have limited initial adhesion due to the hypercapsule phenotype that could conceal these fimbriae [51].

The virulence of *K. pneumoniae* is boosted by a number of outer membrane proteins (OMPs), including OmpA, murein lipoprotein (LppA), and peptidoglycan-associated lipoprotein (Pal) [52]. Similarly, the downregulation of two important porins, OmpK35 and OmpK36, is responsible for resistance to antibiotics and the sacrifice of the bacteria [53,54]. The loss of a new porin, KpnO, can result in increased antimicrobial resistance and reduced virulence in NTUH-K2044 [55]. However, it is yet unclear what mechanisms underlie the decreased virulence caused by the absence of these OMPs.

The type 6 secretion system (T6SS), a novel virulence-related factor, has been discovered in hvKP strains recently [56]. Wild-type NTUH-2044 is more virulent than *Salmonella typhimurium*, *Escherichia coli,* and T6SS-null *K. pneumoniae* in a T6SS-dependent way, and T6SS occurs more frequently in *K. pneumoniae* strains that cause liver abscess than in strains that colonize the intestines [56,57], indicating that T6SS may increase the likelihood of hvKP survival. Furthermore, it is hypothesized that T6SS plays a role in iron import in hvKP as the genes responsible for iron absorption mechanisms are located close to T6SS-related genes (*tssD* in Kp52.145) [58].

Apart from those mentioned above, some virulence factors, such as *moaR*, *mrk* fimbriae, *kva15* regulators, and *kvgAS* signaling systems, have undergone experimental validation in disease-modeling mice [59]. However, it is likely that the virulence factors discovered in these numerous mouse models using diverse strains of hvKP are only a small portion of the genes that hvKP uses to infect healthy hosts. To enhance hvKP diagnostics and find new antibacterial targets, a comprehensive picture of virulence determinants is critically required.

2.5. Definitions of hypervirulence in CR-hvKP

Numerous vital virulence factors that promote the pathogenicity of CR-hvKP have been identified. Currently, the methods for determining the hypervirulence of CR-hvKP are almost consistent with the methods for determining hvKP. Some virulence traits are thought to be hallmarks of hypervirulence. A clinical definition of the hvKP-associated invasive liver abscess syndrome put forth by Siu et al. [60] states that the syndrome results in a liver abscess with extrahepatic consequences, particularly necrotizing fasciitis, endophthalmitis, or central nervous system involvement [60].

However, defining hypervirulence purely based on clinical characteristics can be challenging. There is an overlap of several clinical symptoms, and this is made more challenging by the fact that hvKP can induce nosocomial and healthcare-associated infections similar to cKP [61]. The FDA-approved test, the string test, for use in the clinical microbiology laboratory cannot distinguish between hvKP strains and cKP strains [34], and some non-K1/K2 strains cause pyogenic liver abscess, endogenous endophthalmitis, and other related conditions [62]. The hvKP strain is well defined by its virulence gene repertoire [2]. The molecular definition of hvKP strains is usually based on the presence of several predictive biomarkers we mentioned above (e.g., *rmpA* and *iucA-D*). Aerobactin is the main siderophore generated by hvKP strains and is essential for enhancing virulence both *in vitro* and *in vivo*, indicating that siderophore-related encoding genes, particularly *iucA*, are important markers for hvKP identification [4,43]. Additionally, enhanced capsule synthesis, which is mediated by *rmpA* or *rmpA2*, also contributes to the hypervirulent phenotype [24,43]. Therefore, a combination of markers to define hypervirulence was put forward: *rmpA* or *rmpA2*, and/or *iuc* [30], and genotype, clinical features, and phenotypes are considered in the current definition of hvKP. For example, Liu and Guo [63] used HMV and the presence of aerobactin to define hvKP. Siderophore production greater than 30 μg/ml and *peg-344, iroB, iucA, rmpA,* and *rmpA2* can distinguish hvKp from cKp strains [43]. Additionally, although impractical for the clinical microbiology laboratory, murine, but not *Galleria mellonella*, models are better suited for assessing the hypervirulence phenotype [43,64].

The multiple definitions mentioned above have produced a murky picture of CR-hvKP infection prevalence, detection, and diagnosis. In our opinion, a comprehensive definition of hypervirulence for CR-hvKP should incorporate hypervirulence phenotype in the murine sepsis model, the corresponding virulence gene genotype (e.g., *rmpA* and *iucA*), and clinical symptoms of metastatic infection. However, the microbiological identification of CR-hvKP strains in clinical laboratories remains challenging. As more hvKP-specific genes are discovered, additional markers may also become workable substitutes or even more precise. Therefore, sustained efforts are urgently needed toward establishing a unanimous definition of hypervirulence for CR-hvKP.

3. Resistance mechanism of CR-hvKP

The evolution of CR-hvKP can be attributed to four main pathways, including synthesis of carbapenemase, downregulation or deletion of outer membrane porins, activation of the efflux pump

system, and altered biofilm components. These mechanisms are briefly reviewed in this section.

3.1. Carbapenemases for extensively drug-resistant

Extended-spectrum beta-lactamases (ESBLs) are plasmid-encoded enzymes that hydrolyze β-lactam antibiotics, and the plasmid frequently encodes additional antimicrobial molecules that confer resistance to cotrimoxazole, fluoroquinolones, and aminoglycosides [65]. Although carbapenem antibiotics are effective against ESBL-containing bacteria, the increased use of these antibiotics causes a concomitant emergence of carbapenem-resistant *K. pneumoniae* strains [66]. These strains typically carry carbapenemases, which can break down most β-lactam antibiotics [67]. Ambler [68] categorized carbapenemases into three classes: A, B, and D. All three classes of enzymes can be transferred between bacteria, leading to a high prevalence of drug-resistant strains. It is common for carbapenem-resistant *K. pneumoniae* strains to carry multiple carbapenemase resistance genes. The prevalence of carbapenemase-positive CR-hvKP strains in China is summarized in Tables S1 and S2 (online).

Klebsiella pneumoniae carbapenemase (KPC), the primary class A enzyme, is the most prevalent carbapenemase worldwide [69]. There are numerous subtypes of KPCs, ranging from KPC-2 to KPC-157 [70]. When hvKP acquires bla_{KPC}-harboring plasmids, it can develop into CR-hvKP with traits that increase both virulence and resistance to carbapenem. In China, a remarkable number of KPC-positive CR-hvKP isolates have been reported since 2010, especially among the KPC-2-producing ST11 *K. pneumoniae* strains [71]. The pandemic spread of KPC-2-producing ST11 CR-hvKP strains in China is mainly associated with horizontal transfer mediated by incompatibility group F (IncF) plasmids [72,73].

The primary class B carbapenemase enzymes, commonly known as metallo-β-lactamases (MBLs), are imipenemase (IMP), Verona integron-encoded metallo-β-lactamase (VIM), and New Delhi metallo-β-lactamases (NDM). Oxacillinases 48 (OXA-48) and 181 (OXA-181) are the two most common class D enzymes [74]. These enzymes only partially hydrolyze carbapenem. In China, although KPC-2 is the most predominant carbapenemase, NDM-1/5/7-positive CR-hvKP has also been reported recently [32,75,76]. Additionally, several cases of OXA-232-producing ST15 CR-hvKP have emerged in China, posing a challenge to infection control [77]. Furthermore, China has occasionally reported the occurrence of IMP-positive and VIM-positive CR-hvKP [78,79]. Notably, CR-hvKP strains carrying two or more carbapenemase resistance genes, such as bla_{KPC-2} and bla_{NDM-1}, bla_{KPC-2} and bla_{NDM-5}, and bla_{KPC-2} and bla_{IMP}, are increasingly common [32,80,81]. Gene recombination, rearrangement, and the assembly of fusion plasmids can transfer plasmids encoding these diverse carbapenem-resistance genes, resulting in more intricate resistance mechanisms in CR-hvKP strains [82].

3.2. Decreased expression or loss of outer membrane porins

The OMP is a channel on the bacterial outer membrane for antibiotics. Reduced or loss of OMP expression affects its size, number, or electrostatic properties, hindering carbapenem entry and causing antibiotic resistance [83]. OMPs comprise OmpF, OmpC, LamB, and Pho [84]. *Klebsiella pneumoniae* is characterized by OmpK35, a member of the OmpF group, and OmpK36, a member of the OmpC group. In CRKP, decreased expression levels of *ompK35/36* could affect antibiotic susceptibility [85]. Two CR-hvKP strains in Hubei Province presented high resistance to carbapenems due to the development of ESBL and low expression of *ompK35/36* [86]. Huang et al. [87] detected mutations in *ompK36/37* in a CR-hvKP strain. Wu et al. [88] reported that deletion of the *ompK36* gene lowered the production of OMP and increased carbapenem resistance, showing that carbapenem resistance may be caused by the low expression levels of *ompK35/ompK36*. However, in some studies, *ompK35/ompK36* deficiency appears to play a minor role in carbapenem resistance, possibly serving as a minor cooperative factor alongside ESBLs or AmpC β-lactamases overexpression [89,90]. These findings suggest that the regulatory network involved in carbapenem resistance could be highly complex.

3.3. Activation of the efflux pump system

Efflux of carbapenems via an efflux pump is a key resistance mechanism in Enterobacteriaceae strains. Among CRE, the AcrAB-TolC pump, consisting of AcrA, AcrB, and TolC, is the classic resistance-nodulation-cell division (RND) efflux pump [91]. There are various regulatory variables that control efflux pumps, including *acrR, acrD*, and *rarA* [92–94]. Global regulators like *soxS, marA, rob,* and *ramA* are also crucial AcrAB-TolC regulatory elements [84]. Remarkably, overexpression of *acrAB* can also reduce *ompC* and *ompF* expression [84]. In *K. pneumoniae*, *Klebsiella*-specific efflux pumps such as KpnEF and KpnGH pumps also decrease bacterial susceptibility, giving the bacterium time to adjust to its surroundings [95]. In a 2021 study, a total of 11 CRKP strains causing UTIs, which are often linked to invasive infections associated with hvKP, exhibited *ramA* overexpression and *acrB* upregulation [96]. Among these strains, ST11-KL64, the predominant type of CR-hvKP, was the most prevalent (5/17) [96]. This suggests that the overexpression of efflux pumps is a potential resistance mechanism in CR-hvKP. Unfortunately, the study did not evaluate their virulence phenotype.

3.4. Altered biofilm components

Biofilm, including lipopolysaccharide (LPS), flagella, and type 1 and 3 fimbriae, protects bacteria and helps them survive in harsh environments. Type 1 and type 3 fimbriae, as well as CPS, are the most crucial surface structures in *K. pneumoniae* [48]. CRKP can inhibit flagellar, fimbriae, and pili proteins in response to meropenem stress, promoting biofilm formation and bacterial survival in the presence of drugs [97]. Additionally, among 68 CRKP strains, 77.9% and 22% of isolates were strong and middle biofilm producers, respectively, suggesting a strong relationship between biofilm formation ability and CRKP [98]. Urinary CRKP strains have a higher ability to produce polysaccharides (78.57%) and form biofilm (91.07%) [99]. Therefore, the enhanced biological formation ability may render some *K. pneumoniae* strains even more virulent and antibiotic-resistant. However, some contradictory results have also been reported. Cusumano et al. [100] found that CRKP was 91% less likely to form a strong biofilm, indicating a negative correlation between biofilm formation and carbapenem resistance. Fang et al. [101] also reported that CRKP has a weaker biofilm-forming capacity than cKP due to the absence of the *mrkH* gene, which regulates type 3 fimbriae.

The above complex findings suggest a correlation between antibiotic resistance and biofilm formation, an important virulence factor of hvKP, but the specific biofilm-related mechanisms are unclear. Therefore, further research is urgently required to determine the precise connection between biofilm formation and carbapenem resistance in *K. pneumoniae*.

3.5. Additional resistance to other antibiotics

Considering the poor prognosis associated with CR-hvKP infectious diseases, there is an urgent need for effective antimicrobial

agent therapies. However, additional counterpart resistance issues have emerged during clinical treatment.

Tigecycline, one of the few remaining treatment options for CRKP, has faced additional resistance issues since its approval in China [102]. The resistance mechanisms of CR-hvKP to tigecycline in China primarily involve the overexpression of genes encoding the RND efflux pumps (e.g., AcrAB and OqxAB) or the deactivation of negative regulators (*ramA* and *ramR*) [103,104]. Other factors, including mutated *rpsJ* and plasmid-borne *tet(A)* variant genes, also contribute to tigecycline insusceptibility [105,106].

Colistin, also considered a last line of therapy for CRKP [102], has demonstrated excellent *in vitro* activity against CR-hvKP strains [107]. However, similar to tigecycline, acquired colistin resistance has emerged in Chinese CR-hvKP recently. These mechanisms are related to lipid A modification, including the inactivation of the MgrB protein, overexpression of the two-component regulatory systems (PhoPQ and PmrAB) [104,106,108,109], and the acquisition of plasmid-borne mobile colistin resistance genes (*mcr*), which was first recorded in China [110] but has not yet been found in CR-hvKP.

The use of β-lactam/β-lactamase combo inhibitors, such as ceftazidime/avibactam, has shown high effectiveness against CR-hvKP and provides a hopeful alternative [111,112]. Despite these promising results, the emergence of resistance to ceftazidime/avibactam is a cause for concern. CR-hvKP strains can develop resistance to ceftazidime/avibactam through various mechanisms, including mutations in the bla_{KPC} gene [108]. This underscores the need for continued research and development of novel treatment options to combat the threat of antimicrobial resistance in CR-hvKP infections.

4. Clinical impacts of CR-hvKP

The clinical presentations of CR-hvKP are varied and intricate, including pyogenic liver abscesses, endophthalmitis, and meningitis caused by hvKP, as well as hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections associated with CRKP. Due to its high resistance and pathogenicity, it can often result in poor clinical outcomes. In this section, we briefly discussed the pathogen's clinical characteristics, risk factors, and adverse consequences.

4.1. Clinical features and risk factors

CR-hvKP usually causes healthcare-associated diseases with unique virulence and is, therefore, associated with high morbidity and mortality. The infamous ST11 CR-hvKP isolated in China was generally disseminated prior to initial reporting and led five infected individuals to develop deadly ventilator-associated pneumonia [8]. Since then, a growing number of nosocomial outbreaks of CR-hvKP have been reported [113–117]. Additionally, there have been many reports of patients with liver abscesses [117,118], severe pneumonia [108,119,120], urinary tract infections (UTIs) [96,103,121], bloodstream infection (BSI) and sepsis [103,104,122,123], meningitis [87,124,125], and skin and soft tissue infection [126,127]. CR-hvKP has been less frequently linked to the seeding of other pelvic and abdominal organs, including the kidneys [60], spleen [117], prostate, and scrotum [128]. Recently, there was a report of a patient who died of rare acute purulent pericarditis caused by ST11-KL64 CR-hvKP [129]. In Argentina, the most common infection sites of hypermucoviscous CRKP are the urinary and respiratory tracts (34.2%) [130]. Overall, the presentations of CR-hvKP (depicted in Fig. 2) are diverse and complex, which can simultaneously generate the clinical signs and symptoms of both CRKP and hvKP. At the same time, the total burden of this disease has undoubtedly grown due to the increasing spread of CR-hvKP strains in hospitals.

Underlying comorbidities such as diabetes mellitus and chronic ailments are major risk factors for CR-hvKP infection [129]. Diabetes and age (<65 years) were also independent predictors of septic ocular or CNS complications [131]. Chinese patients have a higher risk of developing K1 capsule-type liver abscesses than Malay, Indian, or Caucasian patients, according to a study of patients in Singapore [13]. However, approximately half of the patients in a collection of 38 instances seen in the United States were not Asian [60]. Therefore, it is still unknown if the origin and prevalence of CR-hvKP in China are due to this population's genetic propensity for the disease or environmental variables.

4.2. Adverse clinical outcomes

CR-hvKP is associated with high morbidity and mortality. Du et al. [132] found that the mortality of CR-hvKP was up to 66.7% in patients with BSI. In patients with postoperative *K. pneumoniae* meningitis, the non-CR-hvKP (1/11) and CR-hvKP (5/9) mortality rates were substantially different, indicating that the convergence of carbapenem resistance and hypervirulence causes high mortality in individuals with *K. pneumoniae* meningitis [125]. *K. pneumoniae* infection in patients on hemodialysis is evolving from the cKP to the CR-hvKP [133]. In oncological patients, strong biofilm-producing CRKP infection, as an independent predictor of mortality, can significantly increase the risk of death [119]. The risk of death is further increased by antimicrobial resistance; in one group of patients infected with isolates that produce carbapenemase, the mortality rate was 100% [8]. Apart from mortality, metastatic illness can have high morbidity. About 70% of individuals with ocular or CNS metastatic illness will experience long-term neurological or visual impairment [134].

The clinical situation raises many issues that are a cause for concern. First, CR-hvKP outbreaks and rapid transmission in medical settings present a significant challenge for infection control. The high level of pathogenicity and resistance frequently leads to subpar clinical results. Second, CR-hvKP appears to be able to colonize the gut and respiratory tract under multiple antibiotic stresses [103,135,136]. These strains pose a high risk of disseminating and have the potential to cause severe infection.

5. Mechanisms of the convergence of hypervirulence and carbapenem resistance

The formation of CR-hvKP is the result of the ongoing evolution of plasmids containing genes for carbapenem resistance or hypervirulence [8], which is a cause for concern. This superbug can not only cause infections that do not respond to antibiotics but also exacerbate the severity of the disease. This evolutionary process can occur through three evolutionary paths: K1/K2 hvKP strains acquiring carbapenem-resistance plasmids [9], CRKP strains acquiring virulence plasmids [8], or a combination of carbapenem resistance and virulence on a single plasmid transmitted by hvKP or CRKP strains [132,137]. In this section, as well as in Fig. 3, we reviewed available mechanism-related studies, excluding case reports (Table S1 online), conducted in China. We dissected and expanded on three fundamental plasmids-related mechanisms underpinning the co-occurrence of carbapenem resistance and hypervirulence phenotypes.

5.1. Acquisition of virulence plasmids by CRKP

The acquisition of virulence plasmids is a crucial mechanism underlying the increased virulence of CRKP, which is the most

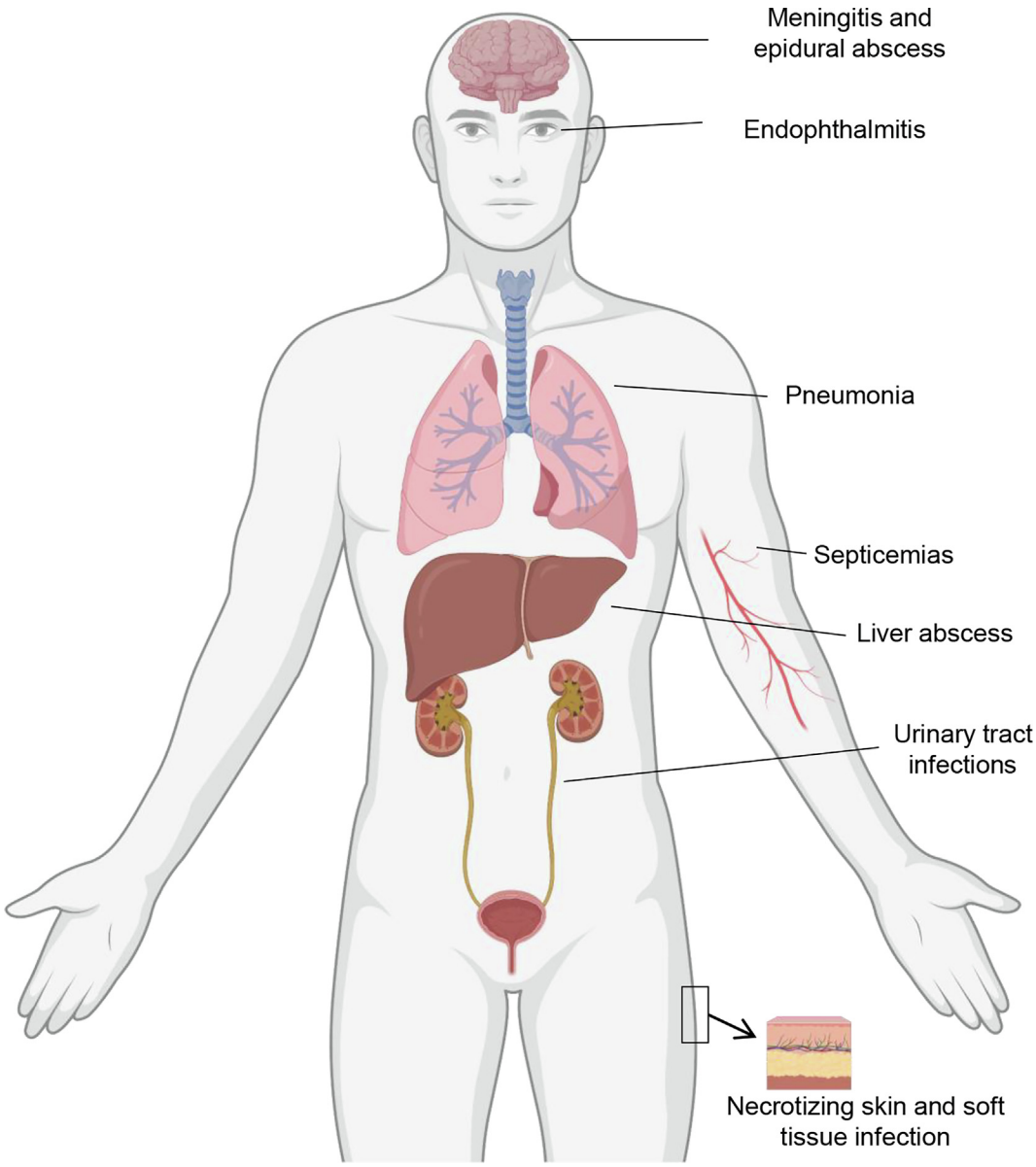

Fig. 2. CR-hvKP infection sites. Common sites of primary and metastatic infections caused by CR-hvKP. (Created with BioRender.com).

prevalent among the three mechanisms. The most well-known virulence plasmids are the 224-kb plasmid pK2044 from *K. pneumoniae* NTUH-K2044 and the 219-kb plasmid pLVPK from *K. pneumoniae* CG43, on which the virulence-associated loci and genes are highly conservatively arranged [2]. Such virulence plasmids are nonconjugative and absent from cKP due to the lack of a complete conjugative module comprising *tra* genes [138]. However, CRKPs acquiring pLVPK-like virulence plasmids have been frequently reported in recent years. Among all the Chinese hypervirulent and carbapenem-resistant *K. pneumoniae*, ST11 CR-hvKP predominates. Notably, ST11-KL64 gradually displaced ST11-KL47 as the major subclone prior to 2015 [139]. There were several plasmids with modest fitness costs in the ST11-KL64 CRKP lineages [140]. Once these CRKP lines acquire the pLVPK-like virulence plasmids, they can quickly spread and cause an outbreak in a hospital [18]. This study also shows that ST11-KL64 CR-hvKP exhibits similar traits to hvKP strains K1 and K2 in terms of mucus thickness, siderophore production, and biofilm formation when compared. Additionally, the hypervirulence of ST11-KL64 hvKP was confirmed in infection models involving human lung epithelial cells and *G. mellonella* [18]. Thus, the method by which the virulence plasmid is transmitted from hvKP strains to CRKP strains is a point for discussion. Dong et al. [109] found the presence of homologous regions between the virulence plasmid and the KPC-2-bearing conjugative MDR plasmid in three ST11 CR-hvKP strains, suggesting that the nonconjugative virulence plasmid might be transferred from hvKP to ST11 CRKP by the co-integrated transfer of these two. This can be explained by the fact that the virulence plasmids themselves cannot spread but are frequently mobilized to access other hosts with the help of other ICEs or conjugative resistance plasmids that encode the conjugation transfer complexes in the same host cell [141]. Recently, Xu et al. [142] demonstrated that with the help of conjugative IncF plasmid, pLVPK-like nonconjugative virulence plasmid from hvKP strains can be transferred to ST11 CRKP strains in various complex forms, including transferring alone, co-transferring with IncF plasmid, and transferring in the form of a hybrid plasmid with IncF plasmid. The type IV secretion system (T4SS) is an important element for the helper plasmid, and

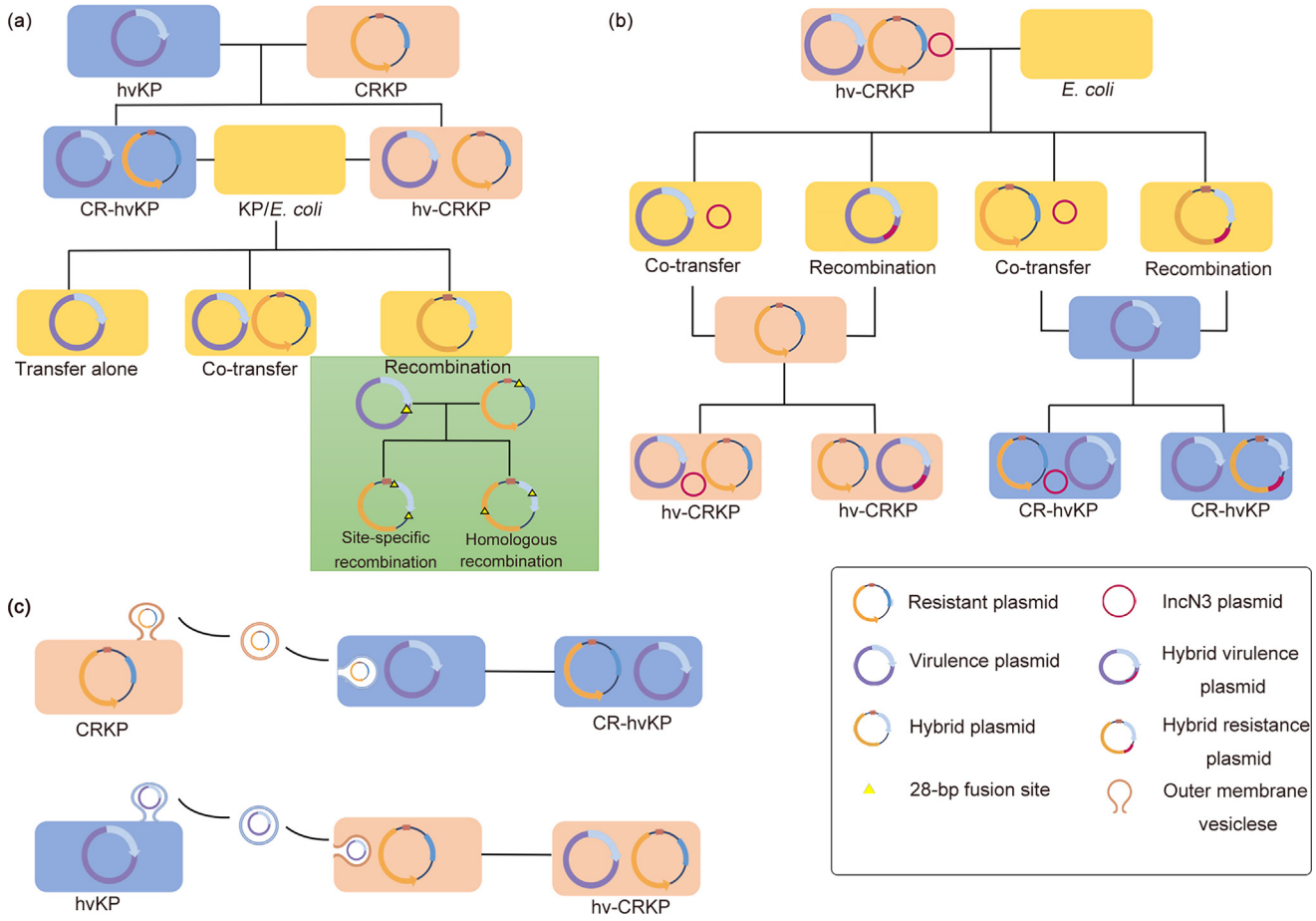

Fig. 3. Proposed mechanisms for the convergence of hypervirulence and carbapenem resistance. (a) Mechanisms of formation of CR-hvKP with the help of conjunctive resistance plasmid (usually IncF plasmid) and further proposed model of the mobilization of virulence plasmid to *Klebsiella pneumoniae* and *Escherichia coli*. (b) Transmission of nonconjugative virulence/resistance plasmids mediated by a self-transferable IncN3 plasmid. (c) Formation of CR-hvKP through Outer membrane vesicles (OMVs)-mediated horizontal transfer.

the helper plasmid can also use the *oriT* of the mobilized virulence plasmid to complete the mobilization [142,143]. Additionally, high extracellular polysaccharide production in *K. pneumoniae* could limit the transfer of virulence plasmids, and *E. coli* could act as an intermediate vector to facilitate this transfer process [142]. Wang et al. [144] reported that coexisting nonconjugative virulence/resistance plasmids can also be mobilized by the IncN3 conjugative plasmid, either directly or via an intermediary *E. coli* in two ways: a hybrid plasmid fused with IncN3 or a cotransfer with the assistance plasmid, IncN3. Furthermore, Wyres et al. [10] demonstrated that multidrug-resistant bacteria are more inclined than hypervirulent strains to create a range of surface polysaccharide sites through chromosomal recombination and acquire virulence plasmids [10]. Outer membrane vesicles (OMVs) play a significant role in the transmission of bacterial virulence factors, leading to inflammation and damage during *K. pneumoniae* infections [145,146]. More importantly, *K. pneumoniae* may develop CR-hvKP because of the coexistence of virulence and carbapenem-resistance genes due to OMVs-mediated horizontal transfer of the virulence plasmid phvK2115 [147]. When hvKP-OMVs carrying virulence genes are transferred to ESBL-producing cKP, the transformed strains display high virulence and multidrug-resistant characteristics [148]. These findings indicate that OMVs-mediated horizontal transfer may serve as a pivotal mechanism contributing to the formation of CR-hvKP.

The pLVPK/pLVPK-like virulence plasmid acquisition can dramatically increase the pathogenicity of CRKP, but survival rates remain unchanged, and the competition ability within and between species enhances even further [8,71,139,149]. However, most CR-hvKP isolates are not hypercapsule-positive, which is an important virulence factor in CR-hvKP [30,31]. The convergence of carbapenem resistance and hypervirulence results from the evolution of the capsule [9]. Therefore, these CR-hvKP may not be as virulent as was initially believed after the loss of the hypercapsule.

5.2. HvKP strains with acquired carbapenem-resistance plasmids

The development of CR-hvKP can be caused by the horizontal transfer of plasmids, transposons, phages, and insertion elements carrying mobile carbapenem-resistance genes from CRKP to hvKP strains. *Klebsiella pneumoniae* can transfer a resistance plasmid to sensitive *Enterobacteriaceae* through OMVs [150]. Another study demonstrated that OMVs derived from CRKP can carry and deliver bla_{NDM-1} to other *K. pneumoniae* strains, such as hvKP [151], which enriches our understanding of the formation mechanism of CR-hvKP. The regional distribution characteristics of carbapenemases indicate that similar regional characteristics can also be seen in the carbapenem-resistance phenotype acquired by hvKP [156]. In China, for example, KPC is the most prevalent carbapenemase, and KPC-positive CR-hvKP strains are also frequently reported [152]. After obtaining virulence plasmids carrying the bla_{KPC-2} gene or a movable DNA fragment carrying bla_{KPC-2}, five K1 hvKP strains transformed into K1 CR-hvKP [153]. Yan et al. [154] isolated an ST23-K1 CR-hvKP strain ZJ27003 from the sputum of an elderly

patient with COPD. This strain contains an IncP1 plasmid, p27003_KPC, carrying bla_{KPC-2}, which can be transferred to *Pseudomonas aeruginosa* PAO1, rendering the receptor resistant to carbapenem. A KPC-2-mediated carbapenem-resistant ST36 hvKP clinical isolate was described by Feng et al. [155]. Similarly, Liu et al. [81] presented an NDM-1 and KPC-2 coproducing ST86-K2 CR-hvKP strain containing four plasmids. Apart from an IncHI1/IncFIB virulence plasmid that is identical to pLVPK, the strain also acquired two carbapenemase-producing plasmids, including a bla_{NDM-1}-carrying IncN plasmid and an IncFIIK plasmid carrying KPC-2 [127]. Notably, a study reported a tigecycline-resistant and IMP-4 carbapenemase-producing CR-hvKP isolate, XH210, recovered from human blood [156]. The ST17-K38 serotype and the conjugative resistance plasmid pXH210-IMP with the bla_{IMP-4} gene were used to identify this CR-hvKP strain. Furthermore, strain XH210 exhibited a hypervirulent phenotype in the *G. mellonella* and mouse infection models but lacked the distinctive characteristics often linked with hypervirulence. This study highlighted the urgent need to increase active surveillance of CR-hvKP strains and identify possibly hypervirulent isolates more accurately [156].

These investigations provide examples of how hvKP clones acquired resistance plasmids that were later shown to be conjugative. It appears that hvKP strains are more prone to acquiring resistance genes than CRKP strains acquiring nonconjugative virulence plasmids. However, evidence suggests the opposite. Several outbreaks of infection caused by CR-hvKP strains, particularly ST11-CR-hvKP, have been documented in a number of Chinese hospitals [8,116]. In contrast, CR-hvKP infections seem to result in fewer hospital outbreaks and are reported less frequently, particularly in China. Numerous factors are possibly to blame for bacteria's survival and predominance in hospitals. Moreover, these CR-hvKP strains may not be hypervirulent and carbapenem resistant, although they have related genes. Relative to the ST11-KL64 CR-hvKP strain, the K1/K2 hypervirulent lineages demonstrate a noteworthy distinction in terms of resistance determinants and antibiotic resistance levels. The K1/K2 lineages exhibit a substantial reduction in the number of resistance determinants and show low-level resistance to antibiotics [18]. Tian et al. [30] showed that the K1 type hvKP strain with HMV lacked corresponding carbapenem resistance initially after acquiring the resistance plasmid but acquired high resistance to carbapenems when the HMV was lost, while the mucoid phenotype was lost in the K2 type hvKP strain that had the KPC plasmid. Furthermore, such CR-hvKP strains attenuated their virulence in mice and larvae *in vivo* after the loss of HMV [30]. Liu et al. [157] recently demonstrated that ST15 CR-hvKP clinical strains with the "red, dry, and rough" morphotype, which was due to a G579D substitution in the BcsA protein, were less virulent than that with typical morphologies.

5.3. The fusion plasmid carrying genes both for virulence and carbapenem resistance

Numerous recent investigations have found hybrid plasmids carrying both resistance and virulence genes in *K. pneumoniae* strains of various sequence types, including cKP clone types, such as ST11, ST101, and ST147 [158,159], and hvKP clone types like ST23 and ST86 [153,160]. Some of these hybrid plasmids are formed by combining conjugative resistance plasmids of the IncF family with virulence plasmids [161]. Yang et al. [162] discovered one such hybrid plasmid in a clinical strain of *K. variicola* strain and verified its capacity for self-transduction. In a different investigation, a clinical *K. pneumoniae* strain underwent homologous recombination between a virulence plasmid and an IncFIA plasmid, resulting in a conjugative hybrid plasmid [163]. In addition, one study identified a hybrid plasmid, pCRHV-C2244, carrying a collection of virulence genes, including *iucABCDiutA*, *iroBCDN*, and *rmpA2*, and a gene bla_{KPC-2}, in an ST11-KL64 CRKP strain in China [137]. This plasmid harbored multiple IS26 sequences that regulated the recombination of the fragments carrying bla_{KPC-2} and virulence genes, as well as the reversal of the large sequence fragment [137]. As a result, the formation of hybrid plasmids is accomplished through recombination between gene fragments that contain insertion elements. Recently, Xu et al. [142] identified the mechanisms for the formation of hybrid plasmids, by which two single-chain segments change at unique fusion sites or undergo homologous recombination.

Apart from the acquisition of virulence plasmids, the integration of these plasmids into bacterial chromosomes can also occur, leading to the evolution of highly virulent strains. The integration process involves the insertion of plasmid DNA into the chromosome and can be mediated by various MGEs. This integration can result in the acquisition of new genetic material and the evolution of new phenotypes that may confer a selective advantage to the bacteria. Recent studies have shed light on the mechanisms involved in the integration of virulence plasmids into bacterial chromosomes. In 2021, whole-genome sequencing of two hvKP strains revealed that hypervirulence genes, such as *iroBCDN*, *iucABCDiutA*, *rmpA/rmpA2,* and *peg-344*, were all put into specific areas of the chromosome [164]. Furthermore, a recent study by Tang et al. [165] in 2023 found that in a CR-hvKP strain, the classic virulence genes were located on a novel T4SS type ICE embedded within the chromosomes. This highlights the diversity of mechanisms involved in the integration of virulence plasmids into bacterial chromosomes and emphasizes the importance of understanding the molecular mechanisms underlying these processes. The integration of virulence plasmids into bacterial chromosomes can have important consequences for public health. The evolved highly virulent strains can be transmitted vertically [164], leading to the emergence of new epidemics. Therefore, understanding the mechanisms involved in the integration of virulence plasmids into bacterial chromosomes is critical for developing effective strategies for the prevention and control of highly virulent and multidrug-resistant strains.

6. Epidemiology and genetic backgrounds

Recently, the incidence of CR-hvKP has been increasing worldwide [7]. China has been identified as the main epidemic area for CR-hvKP, with the largest number of reported cases [166]. To comprehensively assess the epidemiology of CR-hvKP, we conducted a thorough search of PubMed, Web of Science, MEDLINE, CNKI, and Wanfang database for English-language literature published between January 2013 and March 2023, using the following search strategy ("hypervirulence" [All Fields] OR "hypervirulent" [All Fields]) AND ("*Klebsiella pneumoniae*" [MeSH Terms] OR "*Klebsiella pneumoniae*" [All Fields]) AND ("carbapenem resistance" [All Fields] OR "carbapenem resistant" [All Fields]). We filtered and categorized these papers in accordance with the year of isolation, province, isolation type, carbapenem-resistance determinant, virulence determinant, serotype, STs, and isolation number in Table S1 (online), which will be frequently referenced throughout this section.

As shown in Tables S1 and S2 (online), the prevalence of CR-hvKP has been increasing since 2012 in more than half of the Chinese regions with significant regional variation. The highest incidence was observed in the Zhejiang (553 (28.5%) of 1940 isolates), Jiangsu (377 (19.4%) of 1940 isolates), and Beijing (229 (9.7%) of 1940 isolates), followed by Henan (188 (9.7%) of 1940 isolates), Shandong (114 (5.9%) of 1940 isolates), and Hebei (105 (5.4%) of 1940 isolates).

The carbapenem-resistance determinant in CR-hvKP is primarily attributed to the widespread presence of the bla_{KPC-2} gene, with a detection rate as high as 80.7% (1565/1940). The detection rates of NDM, OXA, IMP, and VIM carbapenemase genes were 2.5% (48/1940), 1.8% (34/1940), 0.1% (2/1940), and 0% (1/1940), respectively. Multiple carbapenem-resistance genes were detected in 1.8% (35/1940) of the isolates, and other mechanisms were detected in 0.09% (17/1940) of the isolates. Carbapenem-resistance analysis was not performed in 4.9% (96/1940) of the CR-hvKP strains. Our epidemiological analysis, in concurrence with that of a previous study [71] on CR-hvKP in China, revealed that the majority of CR-hvKP strains belonged to the ST11-KL64 type. ST11 is the most common clone of CRKP in China, with a rate of 80.7% (1565/1940), followed by ST23 at 2.4% (46/1940) and ST15 at 2.3% (44/1940). Serotype KL64 was identified in most CRKP strains (743 (38.3%) of 1940 isolates), followed by KL47 (265 (13.7%) of 1940 isolates). The dominant clone of ST11 CRKP in China has moved from KL47 to KL64 as a result of a combination mechanism, according to phylogenetic analysis of CRKP strains from 2013 to 2017 [166], accompanied by enhanced virulence and more frequent detection of pLVPK-like virulence plasmids.

Moreover, the definition of virulence in CR-hvKP is complex and varies across different studies. However, every study agrees that virulence is a crucial determinant of CR-hvKP. The gold standard for assessing virulence in CR-hvKP remains the murine infection experiment, which, although not yet widely available, has been increasingly used to identify CR-hvKP strains in the last two years.

7. Conclusion

In light of previous reports on the convergence of hypervirulence and carbapenem resistance in *K. pneumoniae*, this review discusses and elaborates on the virulence factors, resistance mechanisms, formation mechanisms, and clinical manifestations of epidemiological characteristics of *K. pneumoniae* in China. In summary, CR-hvKP has spread extensively throughout China and caused serious hospital infections, yet few effective treatments are available. Carbapenem resistance and virulent genetic components are predominantly spread through plasmids. The convergence of hypervirulence and carbapenem resistance occurs through the co-occurrence of virulence and resistance plasmids in the same cell or by the emergence of hybrid plasmids that carry both traits. Additionally, the high mutation and transmission rates of CR-hvKP are primarily attributed to the transfer of plasmids containing mobile genes, as well as the fusion and recombination of plasmids across bacteria. More effective tracking and management of these superbugs in our country can be achieved by understanding the molecular evolutionary processes of resistance and virulence bearing plasmids, as well as the prevalence of CR-hvKP in China. Furthermore, significant efforts are needed to investigate and develop strategies to halt *K. pneumoniae* virulence plasmid proliferation and/or carbapenem resistance in China.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

Danni Pu and Bin Cao contributed to the conception of the review. Danni Pu and Jiankang Zhao searched and analyzed the literature. Danni Pu wrote the manuscript. Bin Cao and Jiankang Zhao conceptualized and led the discussion of the review. Jiankang Zhao, Xianxia Zhuo, and Kang Chang described and revised the manuscript critically. All authors have read and approved the manuscript.

Appendix A. Supplementary materials

Supplementary materials to this review can be found online at https://doi.org/10.1016/j.scib.2023.09.040.

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