Infection and Drug Resistance

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REVIEW

Hypervirulent *Klebsiella pneumoniae*

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Abstract: Hypervirulent *Klebsiella pneumoniae* (hvKP), especially multidrug-resistant hvKP (MDR-hvKP) infections, are distributed globally, and lead to several outbreaks with high pathogenicity and mortality in immunocompetent individuals. This is usually characterized by a rapidly metastatic spread resulting in multiple pyogenic tissue abscesses. To date, even though the explanation of hypervirulent factors of hvKP has been identified, it still remains to be fully understood. The most common key virulence agents of hvKP included (1) siderophore systems for iron acquisition, (2) increased capsule production, (3) the colibactin toxin, (4) hypermucoviscosity, and so on. Several hypervirulence factors have been renewed, and the evolution of MDR-hvKP has been deeply explored recently. We aim to describe a chain of key virulence agents attributed to the lethality of hvKP and MDR-hvKP. In this review, recent advances in renewed factors in hypervirulence were summarized, and potential therapeutic targets are explored. Novel co-existence of hypervirulence agents and multidrug-resistant elements, even the superplasmid, was screened. Superplasmid simultaneously harbours hypervirulence and multidrug-resistant genes and can mobile autonomously by its complete conjugative elements. Research into related immunity has also gained traction, which may cause multiple invasive infections with higher mortality rates than classical ones, such as neutrophil- and complement-mediated activity. The evolution of virulence and multidrug resistance is accelerating. More reliable methods for identifying hvKP or MDR-hvKP must be investigated. Furthermore, it is critical to investigate innovative treatment targets in the future.

Keywords: *Klebsiella pneumoniae*, hypervirulence, multidrug resistant

Plain Language Summary

The evolution of hypervirulence and multidrug resistance in hypervirulent *Klebsiella pneumoniae* (hvKP) is aggressive, which greatly threatens public health and poses a grand challenge to existing therapeutic strategies. Therefore, we summarize the advances in novel ideas for hypervirulence agents and explore potential therapeutic targets. Moreover, the novel co-existence of hypervirulent agents and multidrug-resistant elements and related immune reactions were screened in this review.

Introduction

The acute, fatal clinical syndrome of *Friedlander's pneumonia* was first reported in 1882.[1] Initially, the offending pathogen, which could cause infection at multiple sites, was recognized as *Friedlander's bacillus*. Eventually, it was defined as *Klebsiella pneumoniae* (*K. pneumoniae*), classified under the *Enterobacteriaceae* family. Presently, classical *K. pneumoniae* (cKP) and hypervirulent *K. pneumoniae* (hvKP) are the two main pathotypes of *K. pneumoniae* spreading globally. The main features of the two pathogenic bacteria are shown in Table 1.

HvKP was initially identified in 1986, affecting individuals with a series of episodes of liver abscesses linked to severe endophthalmitis.[2] Although the symptoms of hvKP are nonspecific, hvKP strains can cause serious infections, even mortality in immunocompetent individuals, which is characterized by a rapid metastatic spread resulting in multiple invasive infections, especially, pyogenic tissue abscesses.[3] HvKP infections are geographically widely distributed, particularly in East and Southeast Asia, and pose an emerging threat to Europe. Moreover, the incidence of carbapenem-resistant hvKP (CR-hvKP) infection has steadily increased since it was first reported in 2015.[4] By exploring 13,178

Received: 25 May 2023
Accepted: 3 August 2023
Published: 11 August 2023

Table 1 Primary Features of cKP and hvKP

Characteristics	hvKP	cKP
Hypervirulence-encoding genes	*iro, iuc, iut, irp, fyu, clb, rmp*, etc.	
HMV	Always (+)	(-)
Age of patients	All ages	Often older
Immune status of patients	Immunocompetent or immunocompromised	Immunocompromised
Infection	Community	Nosocomial
	Polymicrobial	Monomicrobial
	Often multiple sites	Usually single sites
Metastasis	Common	Uncommon

Abbreviations: cKP, classical *Klebsiella pneumoniae*; hvKP, hypervirulent *Klebsiella pneumoniae*; HMV, hypermucoviscosity.

K. pneumoniae strains worldwide, 7.8% of the strains were found to be CR-hvKP, which can cause outbreaks globally.[5] According to a major study in China, 36% of the screened carbapenem-resistant *K.* pneumoniae (CR-KP) carried hypervirulence factors.[6]

HvKP strains, especially the multidrug-resistant hvKP (MDR-hvKP) clones, threaten public health seriously and make infection control very challenging. Even though the explanation of hypervirulent factors of hvKP has been identified, responsible agents are still not specific enough. Therefore, we review the advances in renewed agents of hypervirulence, summarize the novel co-existence of hypervirulent and multidrug-resistant elements, and explore the potential therapeutic targets in this article.

Hypervirulent Factors of hvKP

Typical *K. pneumoniae* genomes primarily included core and accessory genes. The core genes, conserved in all the members of this species, harbour a subset of basic factors for pathogenicity, including siderophore enterobactin, fimbriae, multifarious capsular polysaccharide (K antigen), and lipopolysaccharide (LPS) (O antigen). However, the accessory genes encoded several virulence factors.[7]

A chain of virulent agents has been studied which may be attributed to the pathogenicity of hvKP, such as capsules, siderophores, and LPS.[8] Genetic elements on chromosomes, large virulence plasmids, or both, are responsible for encoding the determinants of hypervirulence.[9] Clinicians can use these biomarkers to identify hvKP or MDR-hvKP strains. The evolution of hvKP is always triggered by some important factors, such as (1) the enhanced ability to acquire iron and (2) the increased production of capsule or colibactin toxin.

Siderophore Systems for Iron Acquisition

It is hard for bacteria to metabolize without iron. When encountering a low iron environment, siderophores are synthesized to acquire iron for the growth of bacteria.[10] Siderophores play a key role as pathogenicity agents in bacteria, such as Pseudomonas aeruginosa, Staphylococcus aureus, and Yersinia pestis.[11–13] Furthermore, several new hvKP factors promote siderophore synthesis to obtain more iron, which may increase the lethality of hvKP.[14] Multiple hvKP strains are shaped by isogenic mutants in different siderophore biosynthetic pathways.

The core gene of siderophore in *K. pneumoniae* was *ent*, translating enterobactin and the accessory or acquired genes included *ybt, iuc*, and *iro*, which interpret the yersiniabactin, aerobactin, and salmochelin, respectively.[8] In murine models, the above three accessory systems enhance virulence. Specifically, the *iuc* and *iro* genes are highly expressed in hvKP but scarcely exist in cKP.

The *iuc* and *iro* genes are located on plasmids, which are frequently accompanied by other regulators, such as *rmpA, rmpA2, and rmpC*.[5] Takashi Matono et al, by analyzing 17 hvKP strains, revealed that most of the capsular polysaccharide types of hvKP were K1 genotypes and highly expressed rmpA and aerobactin.[15] Four proteins, encoded by the *iucABCD* operon, take charge of aerobactin biosynthesis. Among them, the *iucB* gene plays a dominant role in augmenting the virulence of *K. pneumoniae*.[16] Aerobactin accounts for more than 90% of the siderophore production.

A mutant strain can grow well without the other three siderophores. To date, aerobactin synthesis proteins have been identified as the most promising antivirulence target of hvKP.[17]

Capsule Production

The capsules are encoded by *wzi, wza, wzb, wzc, wzx*, and *wzy*.[18] There are a variety of capsule types in the *K. pneumoniae* family. K1 and K2 are the main capsule types in hvKP strains. Also, over-expression of the capsule is closely related to virulence. The number, architecture, serum resistance, and virulence of capsules in *K. pneumoniae* are complex and involve a net of regulatory factors (such as MprA/KvrB, SlyA/KvrA, and the Sap ABC transporter)[19] and environmental cues, such as, glucose as an environmental signal, which increases capsule production[20] and decreases the cytokine production of PBMCs in patients with type 2 diabetes, which may weaken the clearance of infection.[21]

Hypermucoviscosity (HMV) was a key virulent factor and has been used to identify hvKP. Compared to non-HMV strains, the HMV strains encountered more immune tolerance to neutrophil extracellular traps.[21] Two novel regulators, the RmpD gene, as a capsule chain length regulator with the help of Wzc[22] and the OmpR, involved in energy production and metabolism,[23] are essential for HMV and hypervirulence. However, in a murine pneumonia model, the hypermucoviscous phenotype remained well when capsule gene expression was impeded by its regulator RmpC. That is to say, capsule synthesis and the hypermucoviscosity phenotype are two distinct biosynthetic pathways.[24] Also, the pathogenic functions for hvKP of HMV and capsular polysaccharide (CPS) are different.[25] Hypermucoviscosity does not fully dependent on capsule overproduction. HMV production also depends on other factors, such as *rmpAC*, 7 iron-acquisition -related genes, and *pagO*.[26]

Dunstan et al characterized the phage RAD2 to lead capsule depolymerase of K2 strains, which inhibits the growth of bacteria in the presence of serum.[27] HvKP strains with high expression of CPS can escape from harmful antimicrobial compounds or bacteriophages (phages). The capsule depolymerase of the hvKP strains may be a potential anti-infective target. The capsular polysaccharide synthesis gene, *wcaJ*, of *K. pneumoniae* plays a great part in polysaccharide synthesis. As initiating enzyme of CPS synthesis, WcaJ regulates the capsule production directly.[28] Using insertion sequence elements to interrupt *wcaJ* in ST23-K1 hvKP resulted in minimal capsule synthesis, which may potentially compromise virulence and a high conjugation capacity with respect to the blaKPC gene, promoting the evolution of CR-hvKP.[29]

Colibactin

Colibactin is another notable feature of hvKP, which is expressed by the gene of *clb* (also referred to as *pks*). Colibactin could crosslink DNA and break the double-stranded DNA of the host cell.[30] Recently, surveys from different areas indicated that the infection of colibactin-producing K. pneumoniae (*pks*-positive K. pneumoniae) was scaled up rapidly.[31] A patient encountered recurrent infections with CG23-I hvKP. The isolates all have colibactin genes; one of the isolates, with duplication of the initiator tRNAfMet gene, grew faster in a poor nutritional environment and exhibited enhanced virulence.[32] The deletion of the *clb*A gene of hvKP abolished colibactin production, which substantially hindered key hypervirulence in meningitis development.[33]

Other Relevant Virulence Factors

There are still many unknown factors regarding the specific characteristics or critical features responsible for hypervirulence factors. The above-mentioned biomarkers are important but have limitations when distinguishing hvKP strains from cKP. Through analysis of 291 ESBL-producing *K. pneumoniae* strains, researchers revealed that the expression of *uge, wabG, rmpA, iucA, fimH, iroB*, and *peg-344* was much more frequent in HMV strains compared to non-HMV strains.[34] Moreover, a cohort study including 85 hvKP and 90 cKP strains reported similar results that support *peg-344, iroB, iucA*, plasmid-borne *rmpA* (*prmpA*), and prmpA2 genes that are special for identifying hvKP strains. The identification of virulence genes of hvKP remains incomplete, particularly the combination of different genes required for maximal virulence.[8] Evaluating the *ybt, clb*, and *iuc* expression, the virulence score system (range from 0 to 5) was used to evaluate the virulence of hvKP.

The exploitation of highly susceptible and specific violent factors for hvKP is still ongoing. Many factors are found to increase the lethality of hvKP through different processes. Novel potential factors, including QseBC[35] and the type VI

system (T6SS)[36] in biofilm formation, Peg-344 acts as a role of an inner-membrane transporter,[37,38] may be involved in hypervirulence roles with worse prognosis.

Evolution of Multidrug Resistance

The initial hvKP isolates were antimicrobial sensitive. However, since the first case was reported in 2015, the prevalence of the multidrug resistance hvKP has increased. Clinicians face greater challenges in treating such infections. The evolution of MDR *K. pneumoniae*, hvKP, and multidrug-resistant MDR-hvKP are shaped by gaining relevant resistance or virulence elements, including conjugative plasmids, integrative conjugative elements (ICE), integrons, insertion sequences, and transposons.[39,40]

Conjugative and Non-Conjugative Virulence Plasmids in hvKP

As for lacking transfer genes, the early virulent plasmids of hvKP are non-conjugative elements.[41] HvKP strains can transfer non-conjugative plasmids by utilizing conjugative plasmids, such as KPC-producing plasmids.[42] Xu et al found that ST11 carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and *E. coli* strains can get the virulence element from hvKP by taking advantage of a conjugative IncF plasmid. The transition of virulence plasmid includes four ways in the study: mobile alone, transfer together with conjugative plasmid, format of hybrid plasmid, and homologous recombination.[43] Additionally, a self-transferable plasmid can mobilize the resistance and nonconjugative virulence plasmids, which facilitate the formation of hv-CRKP (CR-KP gained virulence plasmid) and CR-hvKP (hvKP gained resistance plasmid).[44]

Acquisition of Antimicrobial Resistance Genes

There are several mechanisms by which hvKP strains acquire genes encoding antimicrobial resistance genes. The most common method is to acquire resistance plasmids. Among these, blaKPC-2 and blaNDM-1 were the most prevalent.[4] Another method is integration of resistance genes into the virulence plasmids of the hvKP strains.

Furthermore, during therapy, gene mutations contribute to the evolution of CR-hvKP resistance. A series of CR-hvKP strains were isolated from a male patient with systemic metastatic infections.[45] Tigecycline or colistin resistance was finally developed under the treatment of the two drugs. Mutations of *ramR* and *lon* may account for tigecycline resistance and the *pmrB, phoQ*, and *mgrB* genes mutation for colistin resistance. In a similar mechanism, mutations in *pmrB* and *phoQ* genes and insertion mutations in *mgrB* could result in polymyxin B-resistant hvKP.[46]

Acquisition of Virulence-Associated Genes

Another evolutionary path is that multidrug-resistant cKP captures hypervirulence genes from hvKP, which may develop into hv-MDRKP.[47] Through homologous recombination, multidrug-resistant cKP can obtain a virulence plasmid by cointegration with a helper conjugative plasmid or an acquired conjugative plasmid containing virulence genes. An important mechanism is that ICE elements containing virulence factors integrate into chromosomes; also, the acquisition of plasmid containing both virulence and resistance genes is also promising as well.

The interaction between virulent plasmids and MDR plasmids plays a key role in the evolution of MDR-hvKP. The formation of ST11-KL64 hv-CPKP showed that bla (KPC-2) plasmids could facilitate the virulence of plasmids into CRKP.[48] By analyzing 890 *K. pneumoniae* genomes, Tian et al[42] revealed that hv-CRKP strains behaved stronger in vitality in hospital surroundings than did CR-hvKP strains. The transformation of conjugative KPC-producing plasmids promotes the emergence and prevalence of hv-CRKP strains.

Another novel and potential evolution of the coexistence of multidrug-resistance and hypervirulence could be mediated by an outer membrane vesicle, which horizontally conveyed virulence genes from hvKP to MDR.[49]

Superplasmid, found in ST11 (sequence type 11)-K64 CR-HvKP strain, carries both hypervirulence and MDR genes. The complete conjugative elements in the superplasmid created the power of self-transmissibility. The emergence of a superplasmid seriously threatens public health, and control measures are urgently required.[50]

The coexistence of hypervirulence and multidrug resistance components in the same conjugative plasmid has become increasingly common in the evolution of CR-hvKP. A CR-hvKP strain was reported carrying virulence-related

*iuc*ABCD-*iut*A operon for aerobactin and elements for MDR simultaneously.[51] Co-existence of blaOXA-232, rmtF-encoding plasmids, and pLVPK-like virulence plasmid was found in ST15-KL112 K. pneumoniae.[52]

MDR-hvKP strains are undergoing wide dissemination, even resistant to last-line antibiotics. The evolution of MDR-hvKP is complex and harmful to public health. It is critical to explore more potential and comprehensive mechanisms.

Immune Response

Compared to cKP strains, hvKP strains are more immune tolerant.[53] CPS, a distinctive virulence feature of hvKP, allows the bacteria to avoid being killed by phagocytosis or serum factors.[54] Also, reducing or terminating the biosynthesis of CPS may weaken the virulence of *K. pneumoniae* and expose the bacteria to immune activities.[55]

The basic feature of hvKP strains is infection at several locations. It is unclear whether systemic lesions that appear at the time of infection or are caused by subsequent bacterial metastasis. The hvKP can survive in neutrophils, and the lifespan of hvKP-infected neutrophils is up to 24 h.[56] In a mouse CR-hvKP infected model, low-virulence strains were more susceptible to neutrophil-induced killing than the CR-hvKP isolates. Compared to healthy animals, there is more infiltration of neutrophils assembled in spleens and lungs.[57] After hvKP infection, macrophage replication plays an important role in abscess formation. In the hvKP-infected mice model, Wanford et al found that hvKP escaped from phagocyte-induced killing and spawned in macrophages 6 h after hvKP infection. The presence of neutrophil recruitment at infected sites contributes to the formation of abscesses at an early stage of infection.[58]

Conclusion

The fast emergence of hvKP hypervirulence and multi-drug resistance poses a significant threat to public health. The developments in new and reliable ideas of hypervirulence agents, possible therapeutic targets, and novel forms of coexistence of hypervirulent agents and multidrug-resistant elements were discussed in this paper. With the rapid evolution of hvKP, new reliable agents of hypervirulence and multi-drug resistance must be identified, as well as more reliable targets for therapeutic intervention.

Abbreviations

K. pneumoniae, Klebsiella pneumoniae; cKP, classical *K. pneumoniae*; hvKP, hypervirulent *Klebsiella pneumoniae*; CR-KP, carbapenem-resistant *K. pneumoniae*; CR-hvKP, carbapenem-resistant hvKP; MDR-hvKP, multidrug-resistant hvKP; LPS, lipopolysaccharide; HMV, hypermucoviscosity; CPS, capsular polysaccharide; ICE, integrative conjugative elements.

Author Contributions

All authors made a significant contribution to the work reported. All of them not only took part in the conception, design, execution, acquisition of data, analysis, and interpretation of this review but also took part in drafting, revising, and critically reviewing the article. All authors gave final approval of the version to be published and agreed on the journal to which the article has been submitted. Additionally, all authors agree to be accountable for all aspects of the work.

Funding

This work was supported by a program from the Ministry of Science and Technology of China (2022YFC2009804).

Disclosure

All authors declare no potential conflicts of interest relevant to this article.

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