REVIEW

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Future MICROBIOLOGY

Molecular pathogenesis of *Klebsiella pneumoniae*

Bei Li[‡,1], Yuling Zhao[‡,2], Changting Liu[3], Zhenhong Chen[*,3] & Dongsheng Zhou[*,4]

ABSTRACT Typical *Klebsiella pneumoniae* is an opportunistic pathogen, which mostly affects those with weakened immune systems and tends to cause nosocomial infections. A subset of hypervirulent *K. pneumoniae* serotypes with elevated production of capsule polysaccharide can affect previously healthy persons and cause life-threatening community-acquired infections, such as pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia. *K. pneumoniae* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection. This article aims to present the state-of-the-art understanding of the molecular pathogenesis of *K. pneumoniae*.

KEYWORDS
• *Klebsiella pneumoniae*
• pathogenesis • virulence determinants

Klebsiella pneumoniae, a member of the family Enterobacteriaceae, is a rod-shaped, Gram-negative, lactose-fermenting bacillus with a prominent capsule. Typical *K. pneumoniae* is an opportunistic pathogen that is widely found in the mouth, skin and intestines, as well as in hospital settings and medical devices. Opportunistic *K. pneumoniae* mostly affects those with compromised immune systems or who are weakened by other infections. Colonization of the GI tract by opportunistic *K. pneumoniae* generally occurs prior to the development of nosocomial infections, and *K. pneumoniae* colonization can be further found in the urinary tract, respiratory tract and blood [1]. *K. pneumoniae* biofilms that form on medical devices (e.g., catheters and endotracheal tubes) provide a significant source of infection in catheterized patients [2]. Nosocomial infections caused by *K. pneumoniae* tend to be chronic due to the two following major reasons: *K. pneumoniae* biofilms formed *in vivo* protect the pathogen from attacks of the host immune responses and antibiotics [3]; and nosocomial isolates of *K. pneumoniae* often display multidrug-resistance phenotypes that are commonly caused by the presence of extended-spectrum β-lactamases or carbapenemases, making it difficult to choose appropriate antibiotics for treatment [4,5].

At least 78 capsular (K antigen) serotypes have been recognized for *K. pneumoniae* [6–8]. A few serotypes (including predominantly K1 and K2) have a unique hypermucoviscous (hypervirulent) phenotype due to increased production of capsule polysaccharide (CPS), which is recognized as the most important virulence factor of *K. pneumoniae* (see below) and is defined by the appearance of hypermucoviscous colonies grown on agar plates [9,10]. A string test indicates the presence of hypermucoviscosity when a inoculation loop is able to generate a viscous string larger than 5 mm in length by stretching bacterial colonies on agar plate [10]. The degree of mucoidy appears

[1]Department of Dermatology, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China
[2]The 89th hospital of People's Liberation Army, Weifang, Shandong, China
[3]Nanlou Respiratory Diseases Department, Chinese People's Liberation Army General Hospital, Beijing, China
[4]State Key Laboratory of Pathogen & Biosecurity, Beijing Institute of Microbiology & Epidemiology, Beijing, China
*Authors for correspondence: dongshengzhou1977@gmail.com; zhenhong_chen@hotmail.com
‡Authors contributed equally

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to positively correlate with the successful establishment of invasive infections [11]. Nevertheless, whether there is hypersecretion of capsule in the hypervirulent strains remains questionable, as the increased amount of polysaccharide observed might be some exopolysaccharides rather than 'truly organized' CPS sugars.

Hypervirulent *K. pneumoniae* is highly invasive and can affect previously healthy persons, causing life-threatening and often community-acquired infections, such as pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia [9,10]. In particular, serotype K1/K2-caused pyogenic liver abscess, often complicated by metastatic infections, has emerged worldwide in the past two decades [12]. The ability to metastatically spread from one organ to other organs is characteristic of hypermucoviscous *K. pneumoniae* and is uncommon for enteric Gram-negative bacilli in the presence of host immune defense.

To the best of our knowledge, there are no full reviews on the molecular pathogenesis of *K. pneumoniae*. This article presents the state-of-the-art understanding of the virulence determinants of *K. pneumoniae*, with a particular emphasis on hypervirulent *K. pneumoniae*.

Capsule polysaccharide

• CPS gene loci

K. pneumoniae CPS is an acidic polysaccharide generally composed of repeating units of three to six sugars. *K. pneumoniae* CPS is synthesized by the Wzy-dependent polymerization pathway, which is well characterized for *Escherichia coli* group I CPS [13,14], and the *cps* gene clusters commonly consist of genes for sugar nucleotide synthesis, capsule repeat-unit synthesis and capsular repeat-unit assembly and export [15,16]. The full length of the *K. pneumoniae cps* gene clusters ranges from 21 to 30 kb, harboring 16–25 genes (see [15,16] for the gene structure of different *cps* gene clusters). The 5′ terminal regions of all known *K. pneumoniae cps* gene clusters contain six conserved genes (in the following order: *galF*, *orf2*, *wzi*, *wza*, *wzb* and *wzc*) and the 3′ end regions contain a conserved gene *gnd* and are mostly terminated at the *ugd* gene, while the central regions, which encode the proteins for the polymerization and assembly of CPS subunits, are highly divergent [15,16]. Both *wzy* and *wzi* genes are present in all K types, but each of them has a high level of sequence variability among distinct K types. Accordingly, *wzy*-targeting PCR assays have been established to identify K1, K2, K3, K5, K20, K54 and K57 [17,18], and moreover, *wzi* sequencing is able to define the K types of most clinical *K. pneumoniae* isolates [19]. In addition, the capsular typing method based on *wzc* sequencing has been used for the detection of novel capsular types of *K. pneumoniae* [7].

• CPS synthesis & *magA*

CPS synthesis initiates with the assembly of individual sugar-repeat units, which are catalyzed by different glycosyltransferases in a sequential manner [13,14]. The resulting nascent repeats are transferred across the inner membrane by a flippase Wzx and undergo polymerization by a Wzy polymerase in the periplasmic space [13,14]. Further polymerization control and export of mature CPS to the bacterial cell surface occur under the combined action of Wza (an inner membrane tyrosine autokinase), Wzb (an protein-tyrosine phosphatase) and Wzc (an integral outer membrane lipoprotein) [13,14]. *magA* (wzy_{KpK1}) encodes a capsular K1-specific Wzy polymerase [20], but plays no role in lipopolysaccharide (LPS) synthesis [21] and acts as an important virulence determinant in experimental *K. pneumoniae* K1-induced metastatic infections [22].

• *rmpA*/*rmpA2*

Plasmid-borne *rmpA* and its isoform *rmpA2* encode the transcriptional activators of *cps* gene transcription, CPS synthesis and hypermucoviscosity in *K. pneumoniae* K1/K2 [23–25]. In the K2 strain CG43, both *rmpA* and *rmpA2* are activators of CPS biosynthesis and are important for virulence in mice [23,24]. In the K1 strain NTUH-K2044, besides plasmid-borne *rmpA* and *rmpA2,* there is still an *rmpA* paralog on the chromosome, and only plasmid-borne *rmpA* enhances the *cps* gene transcription [25].

• Resistance to phagocytosis

The presence of a thick capsule at cell surface protects *K. pneumoniae* from opsonization and phagocytosis by macrophages [26], neutrophils [27], epithelial cells [28] and dendritic cells (DCs) [29] by blocking binding and internalization **(Figure 1)**. Hypervirulent *K. pneumoniae* K1 shows significantly lower levels of interaction with macrophages compared with nonhypervirulent strains [30], and moreover, the unusual feature of extensive pyruvation of glucuronic

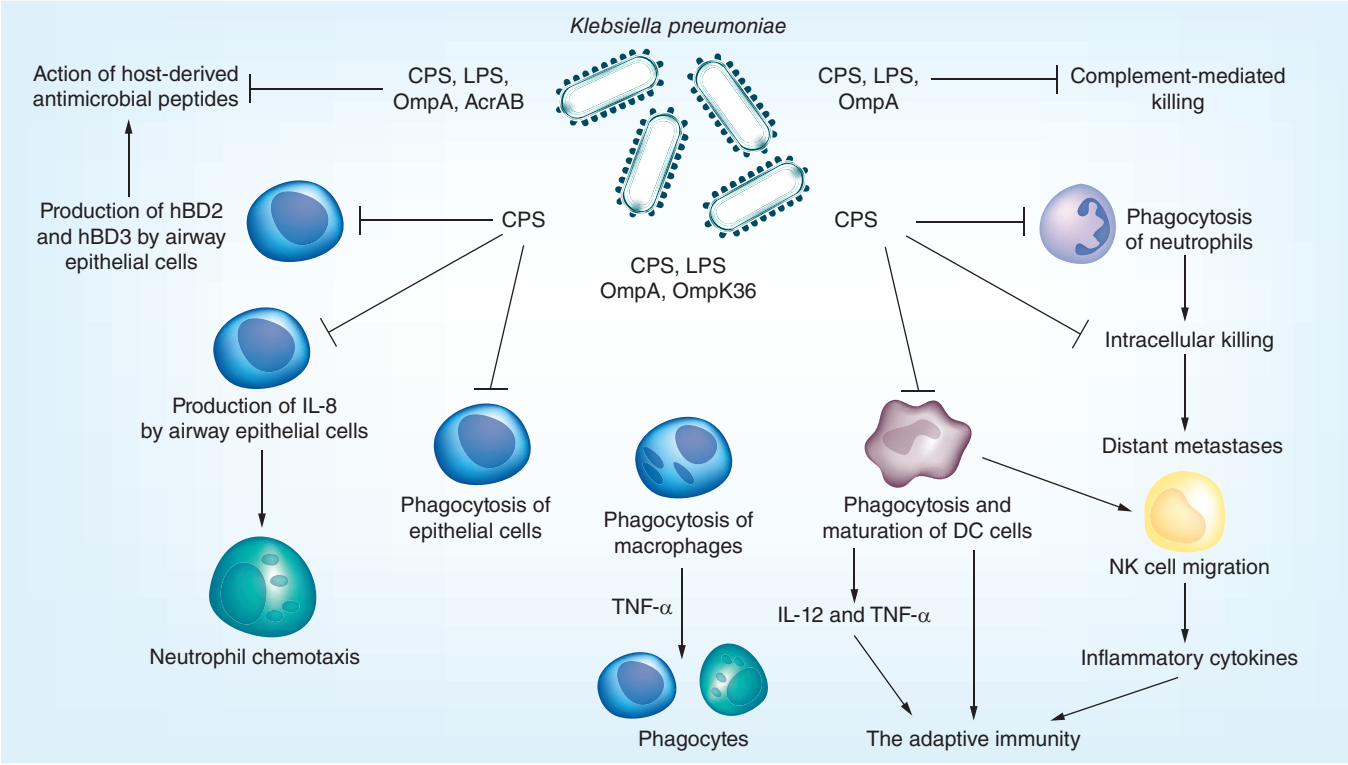

Figure 1. *Klebsiella pneumoniae*-mediated immune evasion. *Klebsiella pneumoniae* employs various surface structures (e.g., CPS, LPS, OmpA, OmpK36 and AcrAB) to evade host immune defenses, enabling the bacteria to resist complement-mediated killing, the action of host-derived antimicrobial peptides and the phagocytosis of epithelial cells, macrophages, neutrophils and DCs. This enables them to escape from neutrophil-mediated intracellular killing of engulfed bacteria, to impair the production of the proinflammatory cytokine IL-8 and antimicrobial peptides hBD2 and hBD3 by airway epithelial cells and to inhibit the maturation of DCs.
CPS: Capsule polysaccharide; DC: Dendritic cell; LPS: Lipopolysaccharide; NK: Natural killer.

acid and acetylation of C_2-OH (or C_3-OH) of fucose in K1 CPS may help *K. pneumoniae* to avoid phagocytosis [31,32]. In addition, hypervirulent K1 *K. pneumoniae*, after being phagocytosed by neutrophils, can steadily escape from neutrophil-mediated intracellular killing and transport the K1 bacteria to distant sites, such as the liver, causing abscess formation **(Figure 1)** [30].

• Suppression of early inflammatory response

Airway epithelial cells produce Toll-like receptors (TLRs) in order to recognize conserved molecules expressed by pathogens, which in turn activate signaling pathways for producing antimicrobial molecules, such as human β-defensins (hBDs; see below) and costimulatory molecules and for releasing cytokines and chemokines **(Figure 1)**. In sharp contrast to the infection of wild-type capsulated *K. pneumoniae* strains that characteristically lacking the above early inflammatory response, avirulent CPS mutants activate a potent inflammatory program [33,34]. Mechanistically, the anti-inflammatory effect of CPS is characterized by inhibition of IL-8 expression through inhibiting TLR2 and TLR4 signaling [33,34] and NOD1-dependent pathways [35].

• Resistance to antimicrobial peptides

CPS at the *K. pneumoniae* cell surface acts as a protective shield against the access of host-derived antimicrobial peptides, and free CPS released from bacterial cells can trap antimicrobial polypeptides in order to reduce the amount of antimicrobial polypeptides reaching the bacterial surface **(Figure 1)** [36,37]. Moreover, sublethal concentrations of antimicrobial peptides in the airway induce *cps* gene expression, which in turn protects bacteria against the action of antimicrobial polypeptides [37]. hBDs produced by airway epithelial cells are potent antimicrobial peptides; hBD1 is constitutively

expressed, whereas the expression of hBD2 and hBD3 is inducible by pathogens and proinflammatory cytokines [38,39]. *K. pneumoniae*-bound CPS decreases the express of hBD2 and hBD3 through preventing TLR-dependent responses and stimulating the expression of CYLD and MKP-1, which act as the negative regulators of hBD expression **(Figure 1)** [40].

• Inhibition of DC maturation

K. pneumoniae CPS can impair DC maturation **(Figure 1)** and thereby reduce the DC-mediated production of pro-Th1 cytokines, such as IL-12 and TNF-α, which will lead to the destructive function of immature DCs during *K. pneumoniae* antigen presentation (thereby impairing T-cell activation), and moreover result in reduced DC-mediated natural killer cell migration [29]. Taken together, inhibition of DC maturation by *K. pneumoniae* CPS allows the bacteria to avoid the host defenses and thus to multiply *in vivo* more easily.

Lipopolysaccharide

LPS comprises three parts: the highly conserved and hydrophobic lipid A anchored in the outer membrane; the highly variable O-antigen as the outermost component of LPS; and the core polysaccharide connecting lipid A and O-antigen.

• O-antigen

At least nine O-antigen groups (O1, O2, O2ac, O3, O4, O5, O7, O8 and O12) have been recognized in *K. pneumoniae* [41]. Biosynthesis of O-antigen is executed by the enzymes encoded by a six-gene *wb* cluster composed of *wzm*, *wzt*, *wbbM*, *glf*, *wbbN* and *wbbO* with respect to their transcriptional direction. The *wb* cluster has a conserved gene organization, but shows high genetic variation in corresponding coding sequences, accounting for the high chemical variability in different O-antigen groups [42]. O1 is the most common serotype among clinical *K. pneumoniae* isolates [41], and it is also more prevalent in hypermucoviscous (invasive) strains than in nontissue-invasive strains [42]. *K. pneumoniae* O-antigen prevents access of complement components to activators (e.g., porins and rough LPS) and thus contributes to bacterial resistance against complement-mediated killing **(Figure 1)** [43], and there is a higher frequency of serum resistance among O1-serotype isolates than among non-O1-serotype isolates [42]. Interestingly, in *K. pneumoniae* O-antigen-deficient strains, CPS protects the microorganism against complement killing **(Figure 1)** [44,45]. As shown in a murine model of septicemia generated by intraperitoneal injection, as well as in a murine model of liver abscess by intragastric injection, the O1 antigen of hypermucoviscous *K. pneumoniae* serotype O1:K1 plays a strong role in virulence by conveying resistance to serum killing and by promoting bacterial dissemination to and colonization of internal organs after the onset of bacteremia [42]. In a murine model of pneumonia caused by hypermucoviscous *K. pneumoniae* serotype O1:K2, both CPS and LPS O-antigens are essential to blood passage of the bacteria and the development of sepsis, but only CPS is involved in the development of *K. pneumoniae*-induced pulmonary infections, because CPS (but not LPS O-antigen) modulates the deposition of C3 and protects the pathogen against human alveolar macrophage-mediated phagocytosis [26]. Nevertheless, O-antigen contributes to lethality by increasing the propensity for bacteremia and not by significantly changing the early course of intrapulmonary infection [46]. The O-antigen of *K. pneumoniae* serotype O5:K57 contributes to *in vitro* adhesion to uroepithelial cells, and also colonization of urinary tracts of rats infected by the transurethral route [43]. As a factor regulating innate immune responses in the lung, surfactant protein D plays an important role in the effective inhibition of the adhesion of *K. pneumoniae* to lung epithelial cells through specific interaction with the mannose-rich repeating units of the O-antigen of this pathogen [47].

• Core polysaccharide

Only two types (type 1 and type 2) of core polysaccharide have been characterized for *K. pneumoniae*, which were synthesized by the products of two different 13-gene *wa* gene clusters [48,49]. The type 2 *wa* gene cluster is composed of *hldD*, *waaF*, *waaC*, *wabK*, *waaL*, *wabM*, *waaQ*, *wabG*, *wabH*, *orf10*, *waaA*, *waaE* and *coaD* [48]. The two *wa* gene clusters differ by only two genes: type 1 has *wabI* and *wabJ*, which encode 3-deoxy-D-manno-octulosonic acid (Kdo) transferase and heptosyltransferase, which are responsible for the incorporation of the last two outer core residues Kdo and L,D-HepI, respectively; type 2 presents the two corresponding genes *wabK* and *wabM*,

which are involved in the transfer of the last two outer core Glc residues [48]. Accordingly, the major structural difference between these two core types occurs at the GlcN substituent in the outer core-proximal disaccharide GlcN-(1,4)-GalA: the Glc*p*N residue of the type 2 core is substituted at the O-4 position by the disaccharide β-Glcp(1–6)-α-Glcp(1), while that of type 1 is replaced at the O-6 position by a Kdo residue or by α-Hep(1–4)-α-Kdo(2) [48,49]. The single-gene mutants of *K. pneumoniae* LPS core synthease genes, such as *waaC*, *waaF*, *wabG* and *wabG*, are greatly attenuated when tested in different animal models [48–51], and show drastically reduced colonization abilities in experimental urinary tract infections in rats [50]. Hypermucoviscous *K. pneumoniae* syntheses type 2 core polysaccharide [52], and the replacement of the type 1 core in a type 2 strain presents lower virulence than the wide-type strain of core type 2 in a murine model infected via the intraperitoneal route [48].

• Lipid A

Nascent core lipid A is synthesized in the cytoplasm by a set of conserved constitutive enzymes, transported by the ABC transporter MsbA and finally anchored in the outer membrane. Occurring during lipid A transportation, covalent modifications of lipid A are catalyzed by various modification enzymes upon environmental stimuli, which is involved in modulating the virulence of a number of Enterobacteriaceae pathogens [53]. *K. pneumoniae* lipid A modification contributes to resistance to host innate defenses, especially including resistance to antibacterial peptides **(Figure 1)**, and the mutation of modification of the genes for this enzyme leads to the attenuation of *K. pneumoniae* virulence when tested in different animal models [54,55]. Lipid A and core polysaccharide, but not O-antigen are required for resistance to phagocytosis by mouse alveolar macrophages **(Figure 1)** [45], which plays an important role in host defense against *K. pneumoniae* [56].

Fimbriae

At least four types of fimbriae, namely type 1 fimbriae [57], type 3 fimbriae [57], Kpc fimbriae [58] and KPF-28 adhesin [59], have been characterized experimentally for *K. pneumoniae*. The former three, as opposed to KPF-28, are synthesized by hypermucoviscous O1:K1 strain NTUH-K2044 with determined complete genome sequences [52], and NTUH-K2044 still harbors six uncharacterized fimbrial gene loci: *kpa*, *kpb*, *kpd*, *kpe*, *kpf* and *kpe* [58].

• Type 1 fimbriae

K. pneumoniae chromosomes contain a conserved fimbria-encoding region that is composed of the regulatory gene locus *mrkHIJ*, the type 3 fimbriae gene cluster *mrkABCDF,* a four-gene interspacing region and the type 1 fimbriae gene locus *fimBEAICDFGHK* [57]. Type 1 fimbriae are thin, rigid, adhesive, thread-like surface appendages on the outer membrane, and the appendages are primarily composed of repeating FimA subunits with an adhesin molecule FimH at the tip [60]. Compared with *E. coli*, the *K. pneumoniae fim* gene cluster has an unique gene *fimK* located behind *fimH*. FimK act as transcriptional regulator with a putative DNA-binding region at its N-terminus and it can specifically bind to a vegetative promoter upstream of *fimA* in order to stimulate *fimA* transcription [61]. A 314-bp region flanked by inverted repeat sequences (*fim* switch), named *fimS*, is located upstream of *fimA*, and moreover, *fimS* mediates the phase-variable expression of *K. pneumoniae* type 1 fimbriae [62], which is very similar to the expression of *E. coli* type 1 fimbriae [63]. *K. pneumoniae* type 1 fimbriae extend beyond the capsule and mediate bacterial adhesion to mannose-containing structures on host cells or on extracellular matrices via the adhesin FimH [64]. *K. pneumoniae* type 1 fimbriae are essential for the initial establishment of urinary tract infection, but have no effect on the ability of *K. pneumoniae* to colonize the intestine or to infect the lung [62].

• Type 3 fimbriae

K. pneumoniae type 3 fimbriae are characterized as 2–4-nm wide and 0.5–2-μm long appendages. *mrkA* encodes the fimbrial subunit, which is polymerized to form the helical fimbrial shaft [65]. The adhesive subunit, with the ability to bind to collagen molecules, is encoded by *mrkD* and located at the tip of the fimbriae [65]. *mrkB, mrkC* and *mrkF* encode the chaperone, usher and scaffolding proteins, respectively, which are responsible for fimbrial assembly/stabilization [65]. Type 3 fimbriae mediate *in vitro* adhesion to epithelial cells and kidney and lung tissues, most likely in a mannose-resistant manner [66]. Type 3 fimbriae act as a major contributor to *K. pneumoniae* biofilm formation, but play no

role in intestine and pulmonary infections [57]. *K. pneumoniae*-induced urinary tract infections are frequently associated with the formation of *K. pneumoniae* biofilms on indwelling urinary catheters. Both type 1 and type 3 fimbriae, functioning in a compensating manner, enhance *K. pneumoniae* biofilm formation on urinary catheters [67]. In addition to type 1 fimbriae, type 3 fimbriae act as another important colonization factor for *K. pneumoniae* biofilm-associated urinary infections due to the indwelling of urinary catheters [68].

• Kpc fimbriae

The Kpc fimbriae are synthesized and assembled by the products of the *kpcABCD* operon, which is highly associated with hypermucoviscous *K. pneumoniae* [58]. A site-specific recombinase-encoding gene *kpcI* together with a promoter element *kpcS* (a 302-bp intergenic DNA region flanked by 11-bp inverted repeats) are located upstream of *kpcA* [58]. KpcA is the major subunit component of Kpc fimbriae, and KpcS in combination with KpcI mediates the phase-variable regulation of Kpc fimbriation [58]. Heterologous expression of *kpcABCD* in a fimbriate *E. coli* makes the recombinant bacterium present Kpc fimbriae, and further confers on it higher biofilm-forming activity, indicating that the Kpc fimbriae may contribute to *K. pneumoniae* biofilm formation [58].

• KPF-28 adhesin

As a polymer of a 28-kDa major fimbrin subunit, KPF-28 is a long, thin and flexible fimbria that is 4–5 nm in diameter and 0.5–2 mm in length, and the structural gene of the KPF-28 major subunit is located on a transferable R plasmid encoding the CAZ-5/SHV-4 β-lactamase [59]. The multidrug-resistant *K. pneumoniae* strains producing plasmid-encoded CAZ-5/SHV-4 and KPF-28 represent a epidemic clone of *K. pneumoniae* in the hospitals in Clermont-Ferrand, France [69]. No KPF-28 expression can be observed in *E. coli* transconjugants harboring the CAZ-5/SHV-4-encoding plasmid alone, and thus, besides the R plasmid, additional factor(s) most likely encoded by the *K. pneumoniae* chromosome are required in order to promote KPF-28 expression [59]. The KPF-28 fimbriae contribute to the adhesion of *K. pneumoniae* to human Caco-2 cell lines, indicating that the fimbriae may be a colonization factor within the mammalian intestine [59].

Outer membrane proteins

• OmpA

It is evidenced that CPS is necessary but not sufficient to attenuate airway epithelial cell-mediated inflammatory responses [35]. OmpA is one of the major outer membrane proteins of Gram-negative bacteria, and it is highly conserved among Enterobacteriaceae. *K. pneumoniae* OmpA, independent of CPS, is important for preventing the activation of airway epithelial cells via acting on NF-κB-, p38- and p44/42-dependent pathways and thus contributes to the attenuation of the airway epithelial cell-mediated inflammatory response **(Figure 1)** [70]. Loss of OmpA makes *K. pneumoniae* more susceptible to antimicrobial peptides, but has no effect on CPS production, which is known to be responsible for resistance to antimicrobial peptides [71]. It is thought that OmpA is involved in the activation of as-yet unknown systems dedicated to ameliorating the cytotoxicity of antimicrobial peptides. OmpA also contributes to resistance to phagoyctosis by alveolar macrophages **(Figure 1)** [45].

• Outer membrane porins

K. pneumoniae produces two major outer membrane porins – OmpK35 and OmpK36 – through which hydrophilic molecules (e.g., nutrients and cephalosporins/carbapenems) diffuse into the bacteria [72]. In addition, *K. pneumoniae* expresses alternative porins, such as KpnO [73] and OmpK26 [74], in order to compensate for the absence of OmpK35/36. Loss of any of OmpK36, KpnO or OmpK26 leads to increased resistance to cephalosporins/carbapenems and reduced virulence in mouse models of acute systemic infections, while loss of OmpK35 has no effect on antibiotic resistance and virulence [45,72–74]. Loss of OmpK36 remodels the surface structure of *K. pneumoniae* and thereby alters the binding of phagocytes, leading to increased susceptibility to phagocytosis and thus an attenuation in virulence **(Figure 1)** [45,72].

• Efflux pumps

K. pneumoniae expresses the efflux pump AcrAB, which contributes to the export of not only antibiotics (e.g., quinolones and β-lactams), but also host-derived antimicrobial agents (e.g., the antimicrobial agents present in human bronchoalveolar lavage fluid and human antimicrobial peptides; **Figure 1**), and AcrAB acts as a determinant of *K. pneumoniae* resistance to host

innate immune defenses [75]. The inactivation of AcrAB not only leads to a multidrug resistance phenotype, but also to a reduced capacity to cause pneumonia in a murine model [75]. The expression of another *K. pneumoniae* efflux pump, namely EefABC, is not related to any antibiotic resistance phenotype, but it confers acid tolerance *in vitro* and high competition potential in the host GI tract [76].

Iron acquisition

Iron is essential for bacterial growth both *in vitro* and *in vivo*. At least 12 distinct iron uptake systems can be identified in *K. pneumoniae* NTUH-K2044, and they can be assigned into four major classes: Fe^{2+} transporter Feo, ABC transporter, hemophore-based uptake systems and siderophore-based uptake systems **(Table 1)**. Of these 12 iron uptake systems, two ABC transporters (Kfu [77] and Sit [78]) and three siderophore-based systems (*Yersinia* high-pathogenicity island [79,80], Iuc [80,81] and IroA [80]) have been shown to be required for the full virulence of *K. pneumoniae*. The *kfu*, high-pathogenicity island, *iuc* and *iroA* regions are highly associated with hypervirulent *K. pneumoniae*, thereby representing horizontally acquired virulence loci during the evolution of hypervirulent variants from opportunistically pathogenic *K. pneumoniae* [82].

Bacterial pathogens cope with the scarcity of iron in their mammalian hosts (in which iron is bound by various iron-binding proteins) through synthesizing small iron-scavenging molecules called hemophores or siderophores. Enterobacteriaceae pathogens, including *K. pneumoniae*, produce a prototypical siderophore called enterobactin, which has the highest iron affinity compared with any other known iron chelators. Mammals have evolved to secrete lipocalin 2 in order to sequester enterobactin, leading to the blocking of siderophore-based iron acquisition as a part of their innate immune response [83]. As a countermeasure, a subset of *K. pneumoniae* isolates with a tendency to cause clinical respiratory or pulmonary infections produces additional siderophores, such as yersiniabactin (a phenolate-type siderophore) and salmochelin (a glycosylated form of enterobactin), in order to rejuvenate the siderophore-based iron acquisition pathways, allowing this pathogen to evade the lipocalin 2-based mechanism of the host innate immune defense [84,85]. Notably, hypervirulent *K. pneumoniae* secrete quantitatively more and biologically more active siderophore molecules than classical *K. pneumoniae*, delineating an additional mechanism by which hypervirulent *K. pneumoniae* increases its pathogenic potential [86].

Nitrogen source utilization

• Urease

Many gut pathogens, including *K. pneumoniae*, can produce cytoplasmic urease in order to hydrolyze urea to ammonia and carbon dioxide as a source of nitrogen for growth. The *ureDABCEFG* operon encodes structural subunits (UreA, UreB and UreC) of the metalloenzyme urease

Table 1. Iron uptake systems in *Klebsiella pneumoniae* NTUH-K2044.

Category	System	Substrate	Gene name	Gene ID
Feo	Feo	Fe^{2+}	*feoABC*	KP1_5110–KP1_5112
ABC transporter	Sit	Fe^{2+}	*sitABCD*	KP1_4347–KP1_4350
	Kfu	Fe^{3+}	*kfuABC*	KP1_1980–KP1_1982
	Fec	Ferric citrate	*fecBDEA*	KP1_3247–KP1_3250
	Yiu	Unknown	*yiuABC*	KP1_1441–KP1_1439
Hemophore based	Hmu	Hemin/hemoprotein	*hmuRSTUV*	KP1_4356–KP1_4362
Siderophore based	Fep-Ent	Enterobactin	*fepA-entD, fes-entF, fepDGC, ybdA, fepB, entCEBA*	KP1_1547–KP1_1546, KP1_1548–KP1_1549, KP1_1553–KP1_1551, KP1_1555, KP1_1556, KP1_1557–KP1_1560
	Fhu	Ferrichrome	*fhuABCD*	KP1_1002–KP1_1005
	IroA	Salmochelin	*iroN, iroBCD*	KP1_3609, KP1_3610–KP1_3613
			iroBCDN	KP1_p028–KP1_p025
	Iuc	Aerobactin	*iucABCD-iutA*	KP1_p319–KP1_p314
	High-pathogenicity island	Yersiniabactin	*ybtPQXS, ybtA-irp2-irp1-ybtUTE-fyuA*	KP1_3586–KP1_3583, KP1_3587–KP1_3593

and accessory nickel-binding proteins (UreD, UreE, UreF and UreG) that are responsible for the incorporation of nickel ions into the active site of the urease enzyme [87]. The inactivation of the *K. pneumoniae* urease-based metabolism of urea will impair the growth of this pathogen in the host GI tract, where urea is abundant [88].

- **Allantoin metabolism**

A 22-kb chromosomal *all* gene locus responsible for allantoin metabolism is highly associated with hypervirulent *K. pneumoniae* [82] and plays an important role in *K. pneumoniae*-induced liver infection [89]. Primary liver abscesses caused by hypervirulent *K. pneumoniae* frequently occur in diabetes mellitus patients with an increased allantoin concentration. The allantoin utilization phenotype described in hypervirulent *K. pneumoniae* elevates its capability to compete for allantoin as a nitrogen source in mammalian hosts [89].

Conclusion

Typical *K. pneumoniae* is an opportunistic pathogen commonly causing nosocomial infections, but a subset of hypervirulent serotypes (including predominantly K1 and K2) due to increased production of CPS affect previously healthy persons to cause life-threatening invasive infections. CPS is the most important virulence factor of *K. pneumoniae*, which plays important roles in resistance to phagocytosis, suppression of early inflammatory response, resistance to antimicrobial peptides, and inhibition of DC cell maturation. Additional *K. pneumoniae* virulence factors include LPS, fimbriae, outer membrane proteins, and determinants for iron acquisition and nitrogen source utilization.

Future perspective

The current understanding of *K. pneumoniae* pathogenesis is mainly derived from studying individual genes, although global screening methods, such as signature-tagged mutagenesis assays [90], have been used for identifying candidates of virulence determination. Huge genetic variability can be found among different types of *K. pneumoniae*, which probably cause dramatic differences in pathogenicity [82]. The regulatory networks governing complex cellular pathways to operate in a concerted manner, which enable the *K. pneumoniae* lifestyle in mammalian hosts, are still poorly understood. Genomics and transcriptomics studies are promising to provide us with deeper understanding of the *K. pneumoniae*–host interactions from a genome-scale view and to identify numerous candidates of virulence-related genes for further hypothesis-based functional characterization.

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EXECUTIVE SUMMARY

Klebsiella pneumoniae-induced infection

- Typical *Klebsiella pneumoniae* is an opportunistic pathogen that commonly causes nosocomial infections.
- Due to their increased production of capsule polysaccharide, a subset of hypervirulent *K. pneumoniae* serotypes (including predominantly K1 and K2) affect previously healthy persons and cause life-threatening invasive infections.

Virulence determinants of *K. pneumoniae*

- Capsule polysaccharide is the most important virulence factor of *K. pneumoniae* that plays important roles in resistance to phagocytosis, suppression of early inflammatory responses, resistance to antimicrobial peptides and inhibition of dendritic cell maturation.
- Additional *K. pneumoniae* virulence factors include lipopolysaccharide, fimbriae, outer membrane proteins and determinants of iron acquisition and nitrogen source utilization.
- The roles of these virulence factors in survival and immune evasion during infection are discussed in this article.

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