Innate Host Defense against *Klebsiella pneumoniae* and the Outlook for Development of Immunotherapies

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Abstract

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative commensal bacterium and opportunistic pathogen. In healthy individuals, the innate immune system is adept at protecting against *K. pneumoniae* infection. Notably, the serum complement system and phagocytic leukocytes (e.g., neutrophils) are highly effective at eliminating *K. pneumoniae* and thereby preventing severe disease. On the other hand, the microbe is a major cause of healthcare-associated infections, especially in individuals with underlying susceptibility factors, such as pre-existing severe illness or immune suppression. The burden of *K. pneumoniae* infections in hospitals is compounded by antibiotic resistance. Treatment of these infections is often difficult largely because the microbes are usually resistant to multiple antibiotics (multi-drug resistant [MDR]). There are a limited number of treatment options for these infections and new therapies, and preventative measures are needed. Here, we review host de-

fense against *K. pneumoniae* and discuss recent therapeutic measures and vaccine approaches directed to treat and prevent severe disease caused by MDR *K. pneumoniae*.

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Introduction

Klebsiella pneumoniae (*K. pneumoniae*; historically known as Friedlander's bacillus) is an opportunistic human and animal pathogen and a leading cause of hospital-acquired infections worldwide. The Gram-negative bacterium is ubiquitous in the environment and found in water, soil, and on plants. Moreover, *K. pneumoniae* is a human commensal and asymptotically colonizes mucosal surfaces of the gastrointestinal tract and to a lesser extent the upper respiratory tract [1]. *K. pneumoniae* infection occurs typically in healthcare settings and the corresponding etiologic pathotype is now commonly referred to as classical *K. pneumoniae* (cKp) [2, 3]. Based on a recent analysis of data from the SENTRY

Antimicrobial Surveillance Program, which spans a 20-year period (1997–2016), *K. pneumoniae* ranks third behind *Staphylococcus aureus* and *Escherichia coli* as a leading cause of bloodstream infections worldwide [4]. In addition to bloodstream infections, cKp commonly presents as urinary tract infection, surgical site infection, and pneumonia [1, 5]. The risk factors for infection include use of indwelling medical devices (e.g., catheters and ventilators) and co-morbidities such as surgery, diabetes, and cancer [5]. In contrast to cKp, some strains of *K. pneumoniae* cause community-acquired infections in otherwise healthy individuals, and the enhanced pathotype is termed hypervirulent *K. pneumoniae* (hvKp) [3, 6]. HvKp was initially described in case reports from Taiwan, and the disease manifested as primary liver abscesses with dissemination [7, 8]. Since the original reports in the 1980s, infections caused by hvKp are increasingly reported worldwide [3]. Several *K. pneumoniae* factors linked to enhanced virulence of hvKp strains reside on mobile genetic elements and contribute to increased capsule production/hypermucoviscosity and iron acquisition [9]. For example, the regulator of mucoid phenotype A (*rmpA* and *rmpA2*) and multiple iron acquisition systems such as *iroBCDN* and *iucABCD* are encoded on a large virulence plasmid named pLVPK [10]. Although infections with hvKp are associated with liver abscesses in otherwise healthy individuals, the clinical spectrum of disease is diverse and the risk factors complex [11]. For example, diabetes is a common risk factor for disseminated hvKp infection [3], and hypervirulent strains have made inroads into healthcare settings [12, 13]. Compared with multidrug-resistant (MDR) cKp strains, the epidemiologic lineage of hvKp is more limited and predominated by 3 clonal groups (CGs) – namely, CG23, CG65, and CG86 [14]. The diverse clinical spectrum of *K. pneumoniae* disease is confounded by the problem of expanding drug resistance in cKp and hvKp (Fig. 1).

Antimicrobial resistance is a global concern and is included as high priority in recent reports by the US Centers for Disease Control and Prevention, the World Health Organization, and the Public Health Agency of Canada [15]. Enterobacteriaceae such as *K. pneumoniae* and *Escherichia coli* are notorious for developing antimicrobial resistance. Cephalosporin and carbapenem antibiotics have historically been a mainstay therapy for treatment of severe infections caused by these pathogenic microbes [16]. However, in the late 1980s and 1990s, there was emergence of *K. pneumoniae* and *E. coli* strains that were resistant to cephalosporin, penicillin, and

monobactam antibiotics due to the acquisition of extended-spectrum beta-lactamases (ESBLs) (Fig. 1). ESBLs are plasmid-encoded enzymes that hydrolyze beta-lactam antibiotics, and the plasmids frequently encode additional antimicrobial molecules that confer resistance to fluoroquinolones, cotrimoxazole, and aminoglycosides [17]. The ESBL-producing cKp strains became an increasing cause of hospital outbreaks and contributed to increased economic burden. Indeed, the SENTRY study indicates ESBL-producing cKp continue to increase in hospitals and the community [4]. Although carbapenem antibiotics are largely effective against ESBL-containing bacteria, use of these antibiotics increased and there was concomitant emergence of carbapenem-resistant *K. pneumoniae* strains [18]. These strains contain a novel class A β -lactamase termed *K. pneumoniae* carbapenemase, which confers resistance to virtually all β -lactam antibiotics. Multilocus sequence type 258 (ST258; assigned to CG258) *K. pneumoniae* are the most prominent *K. pneumoniae* carbapenemase-containing organisms in US hospitals and many other regions worldwide [19, 20]. Carbapenem resistance is also conferred by New Delhi metallo- β -lactamase 1, and thus carbapenem-resistant strains are currently worldwide in distribution. In addition to CG258, there are a number of other important MDR *K. pneumoniae* lineages distributed globally [14]. The genomic population structure of these lineages has been reviewed recently by Wyres et al. [14]

MDR bacteria represent a substantial public health burden and have stretched the thinning antibacterial therapeutic pipeline [21]. Treatment options are often limited. For example, most ST258 isolates are resistant to virtually all β -lactam antibiotics and have decreased susceptibility to aminoglycosides, fluoroquinolones, and many other clinically relevant antibiotics [22]. Antibiotic susceptibility of carbapenem-resistant *K. pneumoniae* is often limited to colistin, tigecycline, and gentamicin, although the prevalence of strains resistant to these compounds has increased recently [23–28]. Resistance to colistin is notable because it can be transferred readily among Enterobacteriaceae by plasmid-encoded *mcr* genes [29–31]. Treatment of carbapenem-resistant *K. pneumoniae* infections with β -lactamase inhibitor combinations such as ceftazidime/avibactam and meropenem/vaborbactam has been successful [32–34], but development of resistance is also a significant concern [35]. There is currently no licensed vaccine for prevention of infections caused by *K. pneumoniae*, and the recent emergence of carbapenem resistance in hyperviru-

Fig. 1. cKp and hvKp. **a** Timeline of the emergence of multidrug resistance in *K. pneumoniae*. **b** Distinguishing features of cKp and hvKp strains. Kp, *Klebsiella pneumoniae*; cKp, classical *K. pneumoniae*; hvKp, hypervirulent *K. pneumoniae*; ESBL, extended-spectrum beta-lactamase; NDM, New Delhi metallo- β -lactamase; Ent, enterobactin; Ybt, yersiniabactin.

lent strains in both community and healthcare settings underscores the need for development of alternative approaches for prevention and/or treatment of infections caused by these organisms [36–40]. Here, we provide a

cursory overview of the host response to *K. pneumoniae* infection and discuss the potential for development of novel immunotherapies and vaccines.

Serum Complement and Complement Resistance

The serum complement system facilitates microbial clearance by augmenting the function of phagocytic cells and/or causing direct lysis of bacteria via formation of the membrane attack complex. Accordingly, defects in the complement system afford *K. pneumoniae* survival in serum. For example, Bain et al. [41] showed that in vitro depletion of alternative pathway factors resulted in increased survival of *K. pneumoniae* in serum. Consistent with these findings, there was increased dissemination of *K. pneumoniae* from experimentally infected lungs to the spleen of mice genetically deficient in factor B or C3 [41]. Although the complement system is a formidable defense against invading microbes, pathogens such as *K. pneumoniae* have evolved strategies to evade killing by serum complement. Notably, a thick capsule polysaccharide (CPS) layer coats the exterior of the bacterium and provides a physical barrier to host molecules present in serum [42]. The CPS consists of a polymer of sugar units known as K antigens and is synthesized by enzymes encoded in the *cps* locus – a set of largely conserved chromosomal genes. Variations in the polysaccharide constituents and structure which result from difference in *cps* loci yield different K antigens, which were classified traditionally by serotype [43, 44]. More recent studies classify *K. pneumoniae* capsules based on the conserved *wzi*, *wzc*, and/or *wzy* genes of the *cps* locus, or by whole genome data [45–49].

To evade killing by serum complement, the CPS masks subsurface structures that trigger complement activation, such as outer membrane protein K36 (OmpK36) [50, 51]. Hence, strains that produce high levels of CPS are typically resistant to serum bactericidal activity [5, 52]. Consistent with these observations, acapsular mutant strains have higher levels of C3 deposition and are more susceptible to killing by serum complement than capsule-positive strains [53]. Indeed, when CPS synthesis was inhibited following treatment of encapsulated *K. pneumoniae* with bismuth dimercaprol, C3b deposition and anti-O1 LPS mAb reactivity increased because subsurface structures were exposed [54]. Recently, Jensen et al. [55] showed that following treatment with serum, susceptible *K. pneumoniae* strains had significant loss of capsule structure and damage to the outer membrane compared with serum resistant strains.

LPS is also known for its ability to protect *K. pneumoniae* against complement-mediated killing [56]. This important bacterial surface structure comprises a lipid A moiety, an oligosaccharide core unit, and a hypervariable

polymer of sugars known as the O-antigen. LPS is classified by the length of the O-antigen as either smooth or rough. Strains expressing a smooth LPS phenotype (smooth LPS) have full-length (high molecular weight) O-antigens, whereas strains with a rough LPS phenotype (rough LPS) have truncated (low molecular weight) O-antigens or lack O-antigens. Generally, strains expressing smooth LPS are less susceptible to bactericidal activity of serum complement than their rough LPS-expressing counterparts [57]. Indeed, studies by Alberti et al. [50, 51] showed that *K. pneumoniae* strains expressing smooth LPS bound less C1q, and there was decreased activation of the classical pathway compared with strains with the rough LPS. Moreover, O-antigens of smooth LPS bind and sequester C3b more distant from the bacterial membrane compared with rough LPS, a phenomenon that hinders the formation of the membrane attack complex [56]. It is notable that rough LPS mutants are susceptible to complement-mediated killing in the presence or absence of CPS [56], an observation that brings into question the unique role of CPS in resistance to serum bactericidal activity.

Although LPS and CPS are considered the factors largely responsible for serum resistance, other *K. pneumoniae* components have been shown to protect against complement-mediated killing. For example, strains deficient in *pal* and *lppA*, genes that encode outer membrane proteins (Omps), are more susceptible to complement-mediated killing than wild-type strains [58]. A recent genome-scale analysis of *K. pneumoniae* identified *rfaH* (an antiterminator), *lpp*, and *arnD* (an LPS modification gene) as genes that play a role in complement resistance [59]. Furthermore, Bachman et al. [60] demonstrated that a mutant *K. pneumoniae* lacking *aroE* (encoding shikimate dehydrogenase) is more susceptible to serum killing than a wild-type strain. Thus, multiple molecules contribute to the serum resistance phenotype of *K. pneumoniae*.

Phagocytes and Host Defense against *K. pneumoniae*

Encapsulated bacteria such as *K. pneumoniae* are often resistant to phagocytosis [61], a characteristic linked to virulence [62]. The resistance to phagocytosis can typically be overcome by host opsonins, most notably a specific antibody combined with a serum complement, and/or via surface phagocytosis [61, 63–65]. Importantly, antibody-mediated phagocytosis has been utilized successfully for vaccine approaches directed to protect against encapsulated bacteria, including *K. pneumoniae* [66, 67].

On the other hand, Wood and Smith first proposed that nonantibody-mediated phagocytosis by adherent leukocytes – so-called surface phagocytosis – is also an important component of the innate host defense against encapsulated bacteria [63]. These authors demonstrated that Friedlander's bacillus phagocytosed by this process is ultimately destroyed within the leukocyte [68]. Not all studies concur with these early findings, and varied results might be explained by different host cell types and experimental assay conditions, or are strain/isolate-specific. For example, a more recent study by Cano et al. [69] reported that an hvKp strain (ATCC strain 43816, a serotype K2 strain used widely for mouse infection studies) alters maturation of the macrophage phagosome, thereby promoting intracellular survival and triggering macrophage-programmed cell death. It is not clear whether this phenomenon is strain-specific, but many bacterial pathogens are known to survive within macrophages.

Studies with a rabbit infection model and anti-polymorphonuclear leukocyte antisera provided strong support to the idea that neutrophils are the primary cellular defense against *K. pneumoniae* [70]. These findings were verified in mouse infection models in which neutrophils were shown to be necessary for defense against hvKp strain 43816 [71–73]. It is also worth noting that mice deficient in neutrophil elastase or myeloperoxidase have enhanced susceptibility to *K. pneumoniae* infection and death [74, 75], results that underscore a prominent role for neutrophils in host defense against *K. pneumoniae*. In humans, neutropenia is a predisposing factor to bacteremia caused by *Klebsiella* spp. [76], and mortality rates are high for bloodstream infections caused by carbapenem-resistant Enterobacteriaceae [77, 78].

Mononuclear phagocytes also have a prominent role in host defense against *K. pneumoniae* infection [72, 79–87]. For example, Xiong et al. [72] demonstrated that CCR2+ inflammatory monocytes contribute to innate host defense against *K. pneumoniae* in the mouse lung. In subsequent studies, the authors showed cross talk between inflammatory monocytes and innate lymphocytes, which involve TNF and IL-17A, leads to enhanced clearance of ST258 from the lungs of infected mice [86]. Consistent with this finding, IL-17A-deficient mice are more susceptible to ST258 infection than wild-type mice [86]. Broug-Holub et al. [82] used mice depleted of alveolar macrophages to evaluate the importance of these host cells following challenge with the hvKp strain 43816. Infected mice lacking alveolar macrophages had significantly decreased survival that was accompanied by more bacteria in the lungs compared to control mice [82]. Al-

though PMN recruitment was not affected, clearance of bacteria from the lungs was impaired [82]. These findings are consistent with a need for macrophage inflammatory protein-2 to promote clearance of the hvKp strain by PMNs, as shown by this group in a separate work [88]. More recently, Ivin et al. [87] reported that increased type I IFN signaling, as a result of a cross talk between alveolar macrophages and natural killer cells, is important for host clearance of strain 43816 in a mouse lung infection model. Notably, increased IFN- γ production by natural killer cells enhances the antimicrobial activities of alveolar macrophages [87].

Bhan et al. [85] used a mouse pneumonia model to show that dendritic cells (DCs) contribute to host defense against *K. pneumoniae* lung infection. In these studies, DCs were recruited to the lungs of mice infected with hvKp (also strain 43816) in a toll-like receptor 9 (TLR9)-dependent fashion, as wild-type mice had fewer bacteria in the lungs and better survival than mice deficient in TLR9 [85]. Although the study used a *K. pneumoniae* infection model, the findings with DCs and TLR9 are likely applicable to lung infections caused by Gram-negative bacteria in general.

Multiple factors can influence contributions of mononuclear phagocytes in the defense against *K. pneumoniae*. Yokota et al. [80] reported that host age has a major impact on the ability of alveolar macrophages to defend against *K. pneumoniae* lung infection in mice. These researchers found that the function of alveolar macrophages rather than a change in the number of these phagocytes in the lungs decreased with age [80]. Subinhibitory concentrations of antibiotics can also increase susceptibility to killing by host macrophages, a phenomenon linked to altered capsule thickness, surface hydrophobicity, and charge [89–91].

Although host phagocytes are essential for defense against *K. pneumoniae*, many *K. pneumoniae* clinical isolates and/or strains are resistant to phagocytosis. The *K. pneumoniae* CPS has long been known to be a major determinant of resistance to phagocytosis [53, 92, 93]. For example, Cortés et al. [53] found that unencapsulated mutant *K. pneumoniae* strains were ingested more readily by human alveolar macrophages than wild-type strains. More recently, Ernst et al. [94] identified a single nucleotide polymorphism in the *wzc* capsule biosynthesis gene that yielded cells with hypercapsule production and resistance to phagocytosis by macrophages. Kobayashi et al. [95] found that there is limited neutrophil phagocytosis of ST258 clinical isolates, a phenotype conferred largely by the CPS. Subsequent work by Diago-Navarro et al.

[96], Kobayashi et al. [97], and Motley et al. [98] demonstrated that phagocytosis and killing of ST258 are enhanced significantly by CPS-specific antibodies [96–98]. These results provide strong support to the idea that the CPS of ST258 can be used as vaccine antigen and/or target.

K. pneumoniae LPS also contributes to resistance to leukocyte phagocytosis. Deletion of *wzm*, the gene involved in LPS O-antigen transport, renders *K. pneumoniae* more susceptible to PMN phagocytosis than wild-type strains [99]. In some *K. pneumoniae* strains, the CPS hinders recognition of LPS by masking the O-antigens and hence limiting phagocytosis. For example, CPS has been reported to attenuate TLR4 signaling in macrophages [100]. However, when the underlying LPS structures were exposed after CPS was disrupted, TLR4 signaling was activated in these host leukocytes [100].

Omps, including Pal, LppA, and OmpA, inhibit neutrophil phagocytosis, consistent with a similar role in serum resistance [58]. It is also noteworthy that *K. pneumoniae* can undergo genetic adaptation to circumvent innate immunity, a process not unlike some of those that result in antibiotic resistance. For example, Ahn and colleagues determined that an ST258 clinical isolate – named KP35 in their study – had acquired new gene orthologs and thereby became resistant to phagocytosis by neutrophils and monocytes [101]. The authors found that this mutant ST258 isolate caused recruitment of immunosuppressive monocytes in a murine lung infection model and in turn altered pro-inflammatory cytokine production and calcium-dependent signal transduction required for killing by phagocytes [101]. The mechanisms underlying this intriguing finding remain incompletely determined and merit further investigation.

Adaptive Immune Responses to *K. pneumoniae*

The activities of serum complement and phagocytic leukocytes are important for the innate immune response to *K. pneumoniae*. Information from the innate immune system is passed on to components of the adaptive immune system usually by antigen-presenting cells [102]. Unlike innate immunity, the adaptive immune response is relatively slow, more specific, and characterized by the generation of immunological memory against reinfection by the same pathogen. Generally, the adaptive immune response can be classified as either cell-mediated, which involves T-cell activation, or a humoral response, which is mediated by B cells and antibodies [103]. Compared

with work in the area of innate immunity, fewer studies have investigated cell-mediated immunity against *K. pneumoniae*, although progress has been made. For example, Lee et al. [104] found that T-helper (Th) type 1, Th2, and Th17 lymphocytes produced IFN- γ , IL-4, and IL-17 following vaccination of mice with *K. pneumoniae*-derived extracellular vesicles. Notably, IL-17 and IFN- γ are important for resolution of *K. pneumoniae* infections [105, 106].

Glycan antigens such as CPS and LPS are generally thought to be less immunogenic and known to induce humoral immune responses in a T-cell independent fashion [107, 108]. This implies that they may not induce the formation of memory cells, and hence any reinfection may appear as a new infection. Nonetheless, several studies have shown that these antigens have the capacity to elicit a strong humoral immune response and the antibodies produced are protective against *K. pneumoniae* infection (discussed below). For this reason, CPS- and LPS-based immunotherapies are viewed as a potential treatment strategy in the wake of the current antibiotic crisis.

Inasmuch as *K. pneumoniae* is a human commensal microorganism, it is not surprising that naturally occurring *Klebsiella*-specific antibodies can be detected in normal human sera. Lepper et al. [109] reported the presence of both CPS-specific (K antigen) and LPS-specific (O antigen) antibodies in the normal human sera of healthy donors. The study also showed that the K-antigen-specific antibodies were vital for clearing *K. pneumoniae* systemic infection and also enhanced phagocytosis of encapsulated *K. pneumoniae* strains by PMNs [109]. More recent studies demonstrated that a serum-sensitive ST258 isolate survived significantly better in IgG-depleted serum, an observation consistent with the presence of naturally occurring antibodies in human serum [110].

Virulence and Animal Infection Models

K. pneumoniae virulence factors are well positioned to provide strong defense against components of the host immune system [5]. CPS is an important and abundant virulence factor of *K. pneumoniae*. For example, K1 and K2 capsule serotypes are primarily associated with hvKp that causes pyogenic liver abscesses [44]. In addition to the immune evasion attributes described above, CPS is important for colonization of the oropharynx and lower gastrointestinal tract [111]. Increased capsule production and hypermucoviscosity in hvKp strains are mediated by a regulator of mucoid phenotype (*rmpA* and *rmpA2*)

[112]. However, the presence of a hypermucoviscous phenotype has also been reported in the absence of these genes [113, 114]. The correlation between CPS production and hypermucoviscosity was investigated further by Mike and colleagues [115]. The authors demonstrated a statistical link between CPS and hypermucoviscosity but also found that certain gene mutations dissociate this relationship. Russo et al. [9] identified *iucA*, *iroB*, *rmpA*, *rmpA2*, and *peg-334* as gene biomarkers that differentiate hvHp from cKp clinical isolates. In general, the presence of *rmpA*, *rmpA2*, and genes encoding 2 siderophores – aerobactin (*iuc*) and salmochelin (*iro*) – are predictors for the hvKp pathotype [116–118]. Siderophores are secreted small molecules used by microorganisms to scavenge iron from the host environment [119, 120]. *K. pneumoniae* aerobactin, salmochelin, and yersiniabactin are encoded on mobile genetic elements, whereas the conserved siderophore enterobactin is encoded within the core genome [121, 122]. Although multiple iron uptake systems can contribute to *K. pneumoniae* virulence [123], Russo et al. [9] demonstrated that aerobactin alone contributes to hvKp survival in human serum and virulence in mouse infection models [124, 125].

Klebsiella spp. can produce multiple surface molecules that facilitate adhesion and attachment to host tissues [126–129]. *K. pneumoniae* type I fimbriae exhibit a mannose-sensitive adhesion phenotype and are closely related to other Enterobacteriaceae type I fimbriae [130, 131]. They are an important virulence factor in urinary tract infections and facilitate binding to the epithelial cells of the urogenital tract [132]. Type I fimbriae are characterized by the bacterial *fim* switch system that is turned “on” or “off” based on cues from the extracellular environment. In liquid broth culture and during urinary tract infection, the switch system is “on,” whereas in static culture or during gastrointestinal tract colonization, the *fim* switch is “off” [132]. Type III fimbriae proteins known as MrkA and MrkD play a distinct role in mediating adhesion and biofilm formation on the extracellular matrix, collagen (MrkD), and abiotic (MrkA) surfaces [133, 134]. Type III fimbriae drive initial cell surface attachment as well as cell-cell adhesion during biofilm formation [129]. In addition to these molecules, Di Martino et al. [135, 136] discovered a *K. pneumoniae* fimbria named KPF-28 and a nonfimbrial adhesin known as CF29K that each promotes binding of *K. pneumoniae* to Caco-2 and Intestine-407 cells in vitro.

Recent genome-scale screens have also led to the identification of bacterial factors that are essential for survival of *K. pneumoniae* in the host and/or contribute to patho-

genesis. For example, Bachman et al. [60] used a transposon insertion site sequencing approach to identify >300 genes in the hvKp strain KPPR1 (a derivative of 43816) that contribute bacterial growth and/or survival in a mouse pneumonia model. More recently, Paczosa et al. [137] used transposon sequencing to identify *K. pneumoniae* molecules that are required for survival in the mouse lung, including several that protect from neutrophil-mediated killing. Such transposon sequencing approaches are becoming more widely used to identify molecules that contribute to bacterial fitness or virulence in vivo [138–145]. These approaches, combined with appropriate animal infection models, can accelerate identification of new targets for therapeutics and vaccines [146, 147].

Designing animal infection models to mimic human disease caused by opportunistic pathogens such as ST258 presents numerous challenges. Healthy mice are relatively resistant to infection caused by ST258, and development of morbidity requires a very high inoculum [110, 148]. This point was demonstrated further by Sequeira et al. [149] who showed a diminished ability of ST258 to colonize the gastrointestinal tract of mice with an established and healthy microbiome. One option is to employ a naturally susceptible animal to model *Klebsiella* infections. Nonhuman primates are naturally susceptible to *Klebsiella*, which manifests primarily as a pulmonary infection [150]. Cynomolgus macaques develop severe lung pathology consistent with bronchiolitis obliterans organizing pneumonia when infected with ST258 [151]. In addition, nonhuman primate lung and airway anatomy are closely related to that of humans. Dumigan et al. [152] used a porcine ex vivo lung perfusion model that closely recapitulated changes observed in human lungs due to infection with *K. pneumoniae*. However, using large animal models has significant challenges and there are additional ethical considerations. Another option to increase animal susceptibility to infection is pretreatment with drugs (typically drugs used for cancer treatment or transplant antirejection drugs) to induce an immunocompromised state. Alternatively, there are murine knockouts and knock-in strains available that mimic various immune system deficiencies. In a recent study by Iwanaga et al. [153], the authors utilized a murine *Rag2*^{−/−} and *Rag2*^{−/−}*Il2rg*^{−/−} pulmonary infection model to evaluate the host immune factors that are important during bacterial infection. Subsequently, the authors developed a more clinically relevant immunocompromised animal model by treating wild-type C57BL/6 mice with the calcineurin inhibitor FK506 (transplant antirejection drug).

Fig. 2. Vaccines and therapeutic approaches for prevention and treatment of *K. pneumoniae* infections. CPS, capsule polysaccharide.

Animal models such as those presented by Iwanaga et al. [153] are a step toward developing adequate models with which to test therapeutic and immunoprophylactic approaches for treating and preventing *K. pneumoniae* infections.

Vaccines and Novel Therapeutic Approaches

Increasing antibiotic resistance among *K. pneumoniae* and the presence of MDR isolates necessitated development of alternative approaches for prevention and treatment of infections, including passive antibody-based therapy approaches and active vaccination (Fig. 2). Antibody-based immunotherapeutics or immunoprophylactic use of vaccines presents several advantages over the use of antibiotics alone. For example, designing antibodies that target only *K. pneumoniae* epitopes could potentially eliminate the pathogen and spare beneficial bacteria that constitute a healthy microbiome [154]. In vitro and in vivo studies indicate the use of specific antibodies dramatically increases uptake and killing of *K. pneumoniae* by immune cells, which consequently leads to improved recovery and increased survival in animal infection models [96–98, 151]. Diago-Navarro et al. [155] generated mAbs specific for the CPS of an ST23 hvKp strain (K1 capsule serotype) and then demonstrated the ability of these mAbs to protect against bacterial dissemination in

a mouse infection model. This research group used a similar approach to test the ability of mAbs specific for CPS glycoconjugates derived from ST258 clade 1 and 2 strains to promote opsonophagocytosis in vitro and reduce bacterial dissemination in a mouse infection model [96]. Cohen et al. [156] used mAbs specific for *K. pneumoniae* LPS to show protection against death in a mouse model of acute pneumonia caused by LPS serotype O1 or O2 *K. pneumoniae* strains. These anti-LPS mAbs protected mice against lethal pneumonia when added before or after inoculation [156]. Studies by Pennini et al. [157] identified human mAbs specific for O1 and/or O2 antigens and found that these antibodies combined with antibiotics protected mice against death caused by MDR *K. pneumoniae* strains. Notably, this study also revealed a relative paucity of human antibody specific for serotype O2 LPS, which may explain in part the higher prevalence of MDR clones that express the O2-antigen (e.g., ST258) [157]. Collectively, these studies underscore the therapeutic potential of mAbs specific for *K. pneumoniae* CPS and LPS.

Bacterial vaccines can be categorized as (i) live attenuated or inactivated/killed vaccines that utilize live attenuated bacteria or dead bacteria, respectively; (ii) toxoid vaccines based on the use of inactivated bacterial toxins; and (iii) subunit (including conjugated) vaccines using only certain parts of the pathogen they are directed against [158]. The use of killed or live attenuated bacteria often offers broad immunity toward a variety of bacterial epi-

topes. As an example, inactivated *K. pneumoniae* is one of 4 bacterial strains used in the Uromune[®] vaccine that currently is undergoing phase 2 clinical trials for use in patients with recurrent urinary tract infections [159, 160]. This vaccine is administered via the sublingual route, and results to date indicate it is safe and effective at preventing recurring urinary tract infections [159, 161]. Moreover, use of heat-killed bacteria in a murine pneumonia model induces a strong Th17-dependent immune response that confers protection during subsequent challenge [162]. *K. pneumoniae*-derived outer membrane vesicles (also known as extracellular vesicles) are intriguing vaccine antigens with potential to elicit broad protective immunity. These spherical nanoparticles are secreted by Gram-negative bacteria and comprise bacterial periplasm and outer membrane that includes LPS and other membrane-associated virulence factors [163, 164]. Preliminary studies have shown that vaccination with outer membrane vesicles, although dose-dependent, can confer complete protection from severe or fatal disease in mouse bacteremia models [104, 165].

When considering more targeted bacterial epitopes for subunit vaccines, *K. pneumoniae* molecules of interest are typically CPS, LPS, or bacterial proteins (Fig. 2). CPS has been used successfully in highly effective meningococcal and pneumococcal vaccines [166, 167]. Hence, it is one of the more obvious targets for immunotherapy and prophylaxis against *K. pneumoniae* infections. Indeed, a multivalent vaccine containing CPS from several different *K. pneumoniae* serotypes was evaluated as a potential vaccine candidate in the 1980s and 1990s, including efficacy and safety testing in humans [66, 67, 168]. Immunogenicity of CPS can be improved significantly by coupling the molecule with a carrier protein, typically an inactivated bacterial toxin. Recently, Feldman et al. [169] used *E. coli* for heterologous expression of CPS from serotypes K1 and K2 hvKp strains (NTUH K2044 and ATCC 43816) and exotoxin A protein from *Pseudomonas aeruginosa* as a means to develop a bioconjugate vaccine. The purified K1- and K2-exotoxin A bioconjugates protected mice against lethal infection caused by K1 or K2 strains [169]. Seeberger et al. used a semisynthetic approach to generate glycoconjugate vaccine antigens that elicit CPS-specific antibodies [170]. Notably, these antibodies were cross-reactive with CPS on the surface of several carbapenem-resistant *K. pneumoniae* isolates tested, underscoring the potential for greater strain coverage [170]. Hegerle et al. [171] reported development of a glycoconjugate vaccine based upon *K. pneumoniae* O-antigens (4 most common O types) and *P. aeruginosa* flagellin. This vaccine utilized

O-antigens chemically linked to flagellin and elicited antibodies that have the potential to confer broad protection against *K. pneumoniae* or *P. aeruginosa* infections.

A well-recognized limitation of CPS-specific vaccines is that there is a relatively high degree of variability in CPS structures among *K. pneumoniae* isolates [44], which presents a challenge for vaccine coverage. Incorporation of more conserved bacterial protein targets may mitigate some of these issues. To this end, Wang et al. [172] demonstrated that antibody generated against MrkA (type III fimbrial protein) reduced bacteria attachment and biofilm formation in vitro and protected mice against lethal pneumonia in a *K. pneumoniae* infection model. Siderophore receptor protein is another conserved protein that has been tested as a potential vaccine candidate. Gorden et al. [173] reported that the use of this protein as a vaccine candidate, to aid with clinical mastitis due to *Klebsiella* spp. in cows, reduced the risk of mastitis by almost 77% if administered before calving. Omps have also shown promise as potential vaccine candidates. A recombinant multi-epitope vaccine (r-AK36) comprising domains from OmpA and Omp36K protected mice from death in a *K. pneumoniae* infection model [174]. Moreover, antibodies specific for r-AK36 conferred significant protection to mice when administered prior to *K. pneumoniae* infection [174]. Taken together, these studies demonstrate the merit of immunoprophylactic and immunotherapeutic approaches for the prevention and treatment of infections caused by MDR *K. pneumoniae*.

Aside from targeting bacterial epitopes directly, several research groups have shown that altering or blocking the function of host immune molecules has potential to aid in more efficient resolution of infection. This strategy is typically geared toward moderating the host immune response to prevent self-propelled damage to the tissue during infection. TLR3 is one such host molecule that has been considered as a therapeutic target. Although typically considered as a viral pattern recognition receptor, TLR3 plays a role in driving an inflammatory response by sensing ds-RNA from necrotic cells [175]. Interestingly, antibody-mediated neutralization of TLR3 increased animal survival in a murine *K. pneumoniae* pneumonia model [176]. Moreover, direct modification of IgG molecules, specifically multiplications of the antibody Fc domain, bolsters antibacterial potential significantly by increasing opsonophagocytic killing of bacteria and increased animal survival in a lethal pneumonia model, compared to the parental antibody [177].

In addition to antibody and vaccine approaches, there is also a renewed interest in the use of bacteriophages

(phages) for treatment of severe bacterial infections [178–181]. Phages are bacterial viruses that infect and, in case of lytic phages, subsequently lyse bacterial cells [182]. Although very sporadic and more as a compassionate use option, phage therapy has been used in patients with a promising outcome [181, 183]. As one of the more recent examples, intravenous phage administration along with oral minocycline was successfully used to treat a prosthetic knee joint infection due to *K. pneumoniae*, with full recovery of the patient [184]. Nonetheless, phage therapy has its own shortcomings. Inherent phage specificity – although advantageous while precisely targeting bacterial strains – can implement constraints when searching for optimal phage treatment [185]. Additionally, bacteria including *K. pneumoniae* are capable of developing resistance toward phage, which potentially can limit the use of mono-phage therapy in treatment of bacterial infections [186]. More work is needed to optimize the utility and therapeutic potential of phage therapy. This includes developing approaches that address the problem of bacterial resistance to phage and translation of phage therapy results obtained from animal infection models to applications in humans [187].

The shrinking repertoire of available antibiotics and paucity of new antibiotics to treat infections caused by MDR bacteria, together with the ability of bacteria to readily acquire antibiotic resistance, have pushed researchers and pharmaceutical companies to consider alternative approaches to treat bacterial infections. Development of a successful vaccine or use of antibody or phage therapy has the potential to reduce antibiotic pressure on bacteria and subsequently would lessen a need for bacteria to acquire antibiotic resistance. Nonetheless, development of a vaccine for *K. pneumoniae* has been (and is) challenging, as there is a relatively narrow market group and a need for precise identification of target populations that potentially could benefit from such a vaccine. Currently, there is no license vaccine available for *K. pneumoniae* in the USA. Each of these novel treatment strategies is still in the early development or pre-clinical stage, and more research is needed to evaluate the potential benefits and applicability of it in humans (Fig. 2).

Concluding Remarks

Prevention and treatment of infections caused by opportunistic pathogens such as *K. pneumoniae* remain a significant challenge in large part because host defense in

the susceptible individual is insufficient to protect against severe disease and because such microbes are often MDR. New therapeutic approaches and vaccines, including targeted mAb therapies and CPS or LPS-specific vaccines, show promise, but more work is needed in these areas. Notably, it will be important to develop animal infection models that more closely mimic human susceptibilities to lineages of *K. pneumoniae* that are opportunistic pathogens.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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