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Klebsiella pneumoniae capsule polysaccharide as a target for therapeutics and vaccines

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ABSTRACT

Carbapenem-resistant (CR) *Klebsiella pneumoniae* has emerged as an urgent public health threat in many industrialized countries worldwide, including the United States. Infections caused by CR *K. pneumoniae* are difficult to treat because these organisms are typically resistant to multiple antibiotics, and the patients have significant comorbidities. Notably, there is high (~50%) mortality among individuals with bacteremia caused by CR *K. pneumoniae*. Given the dearth of new antibiotics, and the recent convergence of multidrug resistance and hypervirulence, there is a critical need for alternative strategies for the treatment of CR *K. pneumoniae* infections. The capsule polysaccharide (CPS) of *K. pneumoniae* has long been viewed as an important virulence factor that promotes resistance to phagocytosis and serum bactericidal activity. Thus, the CPS has been targeted previously for the development of therapeutics and vaccines, although there is no licensed CPS-based vaccine or therapy for the treatment of CR *K. pneumoniae* infections. Here, we discuss immunoprophylactic and immunotherapeutic approaches that have been tested previously for the treatment of *Klebsiella* infections. We also suggest potential strategies to promote development of CPS-based vaccines and therapies for prevention and treatment of CR *K. pneumoniae* infections.

Contents

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1. Introduction

The discovery of penicillin in the early 20th century marked the beginning of the modern antibiotic era, and since that time the discovery and development of new antimicrobial agents has been indispensable for treatment of life-threatening bacterial infections. However, the emergence of antibiotic-resistant bacteria has been concomitant with antibiotic use, thus creating a considerable challenge to prevention and treatment of infectious diseases. Nearly 2 million people are infected with antimicrobial-resistant bacteria annually in the United States, leading to an estimated 23,000 deaths [1]. Although mortality due to antimicrobial resistance is currently low compared with conditions such as heart diseases and cancers (https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm), the global annual mortality rate due to antibiotic resistance is projected to exceed 10 million by 2050 [2]. Moreover, there is an ever-increasing concern of bacteria becoming resistant to all classes of antibiotics, an attribute known as pandrug resistance [3]. *Klebsiella pneumoniae* is of particular concern, because it is among the leading causes of hospital-acquired infections and clinical isolates are frequently determined to be resistant to a broad range of antibiotics [4,5]. The organism also causes community-acquired infections in immunocompromised individuals and/or those with underlying conditions that are risk factors for infection [5].

Klebsiella spp. are gram-negative bacteria that comprise part of the normal gut microbiota. Approximately one-third of humans carry *K. pneumoniae* asymptomatically in their gastrointestinal tract [6]. As such, these commensal microorganisms rarely cause infections in healthy individuals. However, individuals with significant comorbidities are susceptible to life-threatening pneumonia, urinary tract infections, bloodstream infections and surgical site infections caused by *K. pneumoniae* [7,8]. Such infections are therefore prevalent in long-term acute-care facilities and among patients that undergo invasive procedures. Antibiotics, and notably β-lactam antibiotics, are used to treat infections caused by *K. pneumoniae*. Hence, β-lactam resistance in *K. pneumoniae* limits treatment options significantly. *K. pneumoniae* clinical isolates are often resistant to β-lactam antibiotics, primarily because they produce one or more β-lactamases, including extended-spectrum β-lactamases (ESBLs) [9]. These enzymes hydrolyze penicillin and cephalosporin antibiotics, thereby rendering them ineffective. Consequently, carbapenem antibiotics such as meropenem and imipenem became first-line treatment options for infections caused by ESBL-producing bacteria, especially *Enterobacteriaceae* (e.g., *Escherichia coli* and *Klebsiella* spp.) [9]. Inasmuch as antibiotic resistance typically develops under conditions of heavy antibiotic use, and considering *K. pneumoniae* is a gut commensal microbe, it is not surprising that *K. pneumoniae* can acquire carbapenem-resistance. Carbapenem-resistant *K. pneumoniae* strains usually harbor a plasmid-encoded carbapenemase that hydrolyzes all carbapenems and confers resistance to practically all β-lactam antibiotics. The two most prevalent enzymes are known as *K. pneumoniae* carbapenemase (KPC) and New Delhi metallo-beta-lactamase (NDM-1) [10,11]. Of the two, KPC is predominant in the United States and other industrialized countries [8].

Carbapenem-resistant *Klebsiella* spp. are the most common carbapenem-resistant Enterobacteriaceae (CRE) in the United States and are responsible for significant annual morbidity and mortality [1]. The epidemiological success of KPC-producing *K. pneumoniae* (KPC-*K. pneumoniae*) strains has been attributed largely to a strain classified by multilocus sequence typing as ST258 [12]. ST258 accounts for ~70% of the carbapenem-resistant *K. pneumoniae* clinical isolates in the United States and is abundant in many countries globally [7,12,13]. For example, a multicenter analysis of clinical KPC-*K. pneumoniae* isolates from the New York/New Jersey area revealed that 84% of isolates are ST258 [14]. In addition to β-lactam resistance, ST258 strains have decreased susceptibility to aminoglycosides, fluoroquinolones, and many other clinically relevant antibiotics [15]. This multidrug resistance attribute is a major problem for treatment of infections. For example, a carbapenem-resistant *K. pneumoniae* isolate reported in a recent case study of fatal infection was resistant to 26 antibiotics [16]. Last-line treatment options such as colistin and tigecycline have been shown to be successful when administered as combination therapies rather than monotherapy [17]. However, colistin use is associated with side-effects such as nephrotoxicity [18], and considering that patients with KPC-*K. pneumoniae* infections likely have severe comorbidities, colistin-based treatment may not be ideal. Moreover, resistance to colistin in KPC-*K. pneumoniae* strains is emerging rapidly [8]. Although recent combination therapies with β-lactam antibiotics and β-lactamase inhibitors, such as ceftazidime-avibactam [19–21], have been successful for treatment of infections caused by KPC-*K. pneumoniae*, development of resistance remains a concern [21,22].

Thus, there is an urgent need for alternative approaches such as immunotherapy to combat this public health crisis. Here, we review *Klebsiella pneumoniae*-host pathogen interactions in brief, highlighting the role of capsule polysaccharide as an immune evasion molecule and vaccine target.

2. *Klebsiella pneumoniae* virulence molecules and host interaction

The host innate immune system consists of multiple components that provide an early line of defense against invading pathogens. Polymorphonuclear leukocytes (PMNs or neutrophils) are the most prominent cellular defense against invading bacteria and fungi. PMNs are recruited rapidly to damaged and/or infected tissues through host- and microbe-derived chemotactic factors [23,24]. Neutrophils bind and ingest microorganisms by a process known as phagocytosis, which is facilitated by host antibody and serum complement components [25,26]. Ingested microbes are then exposed to numerous microbicidal factors within the phagosome. In addition to neutrophils, monocytes and other leukocytes play an important role in defense against microbial pathogens. For example, it was recently reported that innate lymphoid cells engage in resolution of *K. pneumoniae* pneumonia [27].

Invading microbes are also subjected to numerous soluble extracellular antimicrobial factors present in serum or within tissues, such as serum complement and antimicrobial peptides. These host molecules typically play a key role in defense against bacterial infection. To evade the host innate immune system, bacteria have evolved a repertoire of strategies and/or virulence factors that contribute to their survival in the host. As discussed previously, *K. pneumoniae* is most commonly an opportunistic pathogen and consistent with that lifestyle, produces relatively few virulence factors *per se* [6]. Nevertheless, some strains of *K. pneumoniae* are adept at evading recognition by the host innate immune system and are resistant to phagocytosis. Lipopolysaccharide (LPS), capsule polysaccharide (CPS), siderophores (e.g., aerobactin) and adhesin are some of the factors that enhance survival and virulence of *K. pneumoniae* [6]. LPS (O antigen) and CPS (K antigen) are constituents of the outer membrane of *K. pneumoniae* that are integral for interaction with the host [6,28].

2.1. Lipopolysaccharide (LPS)

K. pneumoniae has lipopolysaccharides (LPS) anchored in the bacterial membrane (Fig. 1). LPS structurally comprises three components – a lipid A component which anchors the entire structure

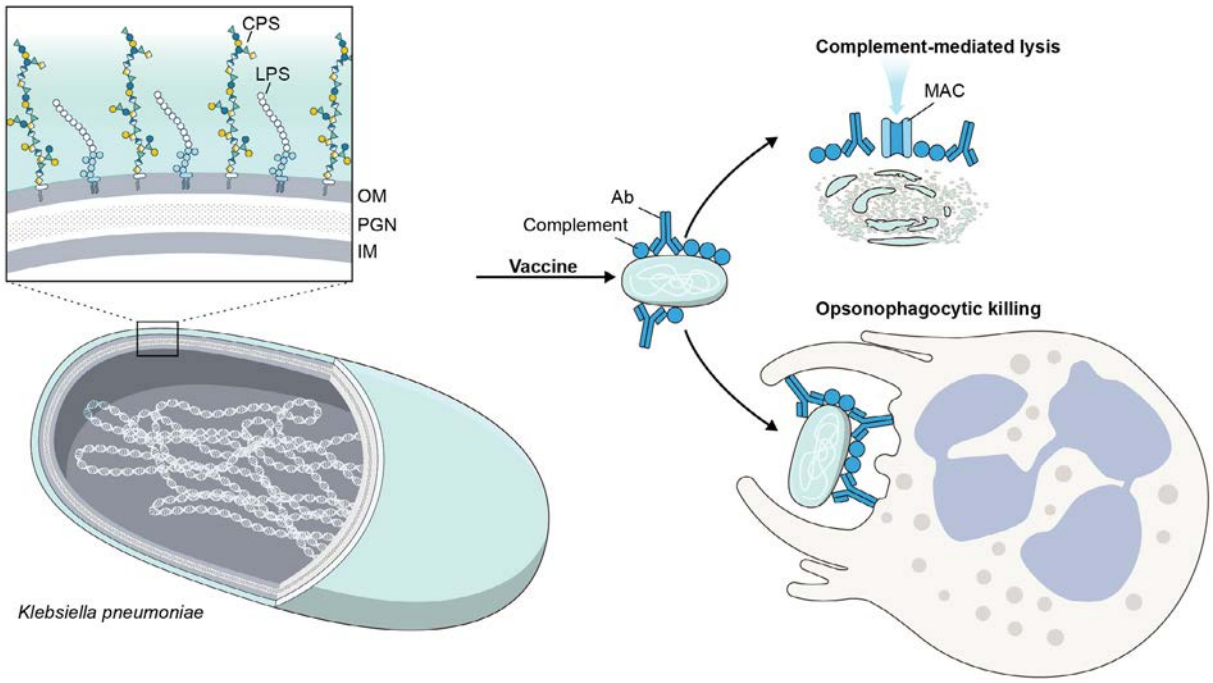

Fig. 1. Immunotherapy approaches utilizing *K. pneumoniae* LPS and CPS. See text for details. CPS, capsule polysaccharide; IM, inner membrane; LPS, lipopolysaccharide; OM, outer membrane; PGN, peptidoglycan.

in the bacterial membrane, an oligosaccharide core, and a terminal side chain called the O antigen. The lipid A component of LPS is a hydrophobic moiety localized in the outer leaflet of the outer membrane and is synthesized by a series of enzymes encoded by the *lpx* gene cluster [29]. Lipid A plays an important role in host recognition of LPS and binds strongly to *Toll*-like receptor 4 (TLR4) leading to potent activation of the immune response [30]. However, lipid A modifications during infection can significantly hinder detection of *K. pneumoniae* by immune cells [31]. The *waa* locus harbors the genes that encode the core oligosaccharide, which links lipid A to the O antigen. The O antigen is covalently linked to the oligosaccharide core by the WaaL ligase encoded by *waaL* [32]. The O antigen consists of a polymer of repeating oligosaccharide units on the outermost part of the LPS structure. The *wb* (formerly *rfb*) gene cluster regulates the synthesis, assembly and transfer of structurally diverse O antigens. The diverse O antigens repeats account for the structural variations of LPS [33].

Nine *K. pneumoniae* O antigens have been identified, and the diversity is conferred primarily by variation in the composition and sequence of sugar monomers [33]. For example, two of the nine identified O antigens, O1 and O2, contain homopolymer galactose units (or galactans). O1 and O2 antigens each contain D-galactan I polysaccharides, but O1 antigens differ in that they have a D-galactan II cap structure [34]. Differentially capped O antigens lead to variation in antigenicity. Recently, Szijártó et al. [35] reported that a variant of D-galactan I, called D-galactan III was predominant among strains of the ST258 lineage and conferred enhanced survival in serum. Although the O1 antigen is the most common serotype [34], Pennini et al. [36] recently reported that the O2 antigen is less immunogenic but predominant among multidrug resistant ST258 isolates. It is feasible that the decreased immunogenicity may contribute to the success of ST258 [36]. The length of O antigens also plays a role in bacterial survival as strains expressing full length O antigens (also called "smooth" LPS phenotype) are less susceptible to complement-mediated killing than those that lack or express truncated O antigens (also called "rough" LPS phenotype). Resistance to complement-mediated killing has been attributed to the ability of LPS—specifically the O antigens—to bind and sequester components of the complement system or prevent binding to the bacterial surface altogether [37,38]. For example, the O antigen has been shown to block C1q binding, thus preventing activation of the classical complement pathway and formation of the membrane attack complex (MAC, C5b-C9), which is bactericidal [39]. Moreover, the MAC fails to form on the surface of bacteria with a smooth LPS phenotype because C3b binds too far from the cell membrane [40]. Depending on the CPS serotype (K antigen; discussed below), some *K. pneumoniae* strains are able to mask the O-antigen. Masking of the LPS hinders detection of the bacteria by the immune system. However, the O1 antigen is mostly surface exposed regardless of the presence of capsule polysaccharides [34,41].

2.2. Capsule polysaccharide (CPS)

Klebsiella pneumoniae produces an acidic capsule polysaccharide that is important for survival in the host [6]. *K. pneumoniae* has been classified historically by capsule (K antigen) serotyping and to date 79 capsule types have been identified [42]. More recently, several groups have proposed a classification scheme for CPS based upon the sequence of conserved *wzi* and/or *wzc* genes within the *cps* locus [43–45]. Whole-genome sequence analysis of ST258 clinical isolates revealed the existence of two distinct subclades rather than a single clone [46]. These ST258 clades are differentiated by a region of hypervariable DNA that includes the *cps* locus. Clade I contains *cps-1* (*wzi29*) and clade II contains *cps-2* (*wzi154*) [46]. The majority of ST258 clinical isolates recovered from patients in the United States and worldwide can be classified as either *cps-1* or *cps-2* [14,47,48].

The *K. pneumoniae* CPS is comprised of repeating sugar units that form a protective layer of material on the bacterial surface (Fig. 1). CPS biosynthesis is dependent on multiple genes that comprise a *cps* locus. Translocation and assembly of CPS onto the bac-

terial surface are regulated by proteins encoded by the conserved genes *galf*, *orf2*, *wzi*, *wza*, *wzb* and *wzc* at the 5′ end of the *cps* locus. Genes involved in sugar biosynthesis, such as rmlA, rmlB, rmlC and rmlD (involved in the synthesis of dTDP-L-rhamnose) and manB and manC (synthesis of GDP-D-mannose), are located at the 3′ end of the locus and are flanked by conserved genes known as *gnd* and *ugd*. The central region between *wzc* and *gnd* contains genes involved in synthesis of CPS repeat units (e.g., *wbaP* or *wcaJ*) and *wzy*, the capsule polymerase that is important for assembly of capsule subunits on the bacterial surface [42]. Variation in nucleotide sequence and number of genes underlies the differences in *K. pneumoniae* capsule types [49].

The CPS is widely known as an important *K. pneumoniae* virulence factor, largely because of its ability to block phagocytosis [50], and severity of *Klebsiella* infections has often been attributed to strains with specific K antigens or CPS types. For example, the CPS of *K. pneumoniae* strains expressing K1 and K2 contributes to invasive community-acquired infections such as pyogenic liver abscesses [51]. Compared with encapsulated strains, unencapsulated *K. pneumoniae* strains are more susceptible to killing by serum complement, and they are more readily phagocytosed and killed by phagocytic leukocytes [52,53]. Also, unencapsulated strains have decreased pathogenicity in mice compared with encapsulated strains [54].

3. Immunotherapy

Bacteria constantly fend off antimicrobial attacks from neighboring and competing species in their natural environs. The defense mechanisms employed can be present naturally or acquired on a plasmid and are often similarly used to inactivate antibiotics during infection. Antibiotic resistance is therefore a natural phenomenon and hence resistance to new antibiotics is inevitable [55]. Fortunately, the human immune system has a unique set of strategies to combat bacterial infections and these strategies do not readily select for resistance in bacteria. Consequently, researchers have long been interested in harnessing the principles of host immunity to combat serious bacterial infections such as those caused by CR-*K. pneumoniae* [6]. Therapeutic approaches including passive and active immunization focus on augmenting the host immune response. For passive immunization, antibodies specific for bacterial antigens are used directly for treatment or prevention of infection. Protection is transient. By comparison, active immunization involves the use of bacteria antigens, such as polysaccharides and/or proteins, to vaccinate the host. The immunized host in-turn develops protection (optimally long-lasting) against the microbe. There are currently no FDA-licensed vaccines for treatment or prevention of *K. pneumoniae* infection. An immunotherapy approach using passive or active vaccines could provide a viable alternative to the use of antibiotics for treatment/prevention of severe *K. pneumoniae* infections.

3.1. Passive immunotherapy

K. pneumoniae CPS is a major cell surface antigen that elicits production of host antibodies. In healthy individuals, naturally occurring *Klebsiella*-specific antibodies contribute to host defense against *K. pneumoniae* by promoting complement deposition and phagocytosis by neutrophils and other phagocytic leukocytes [53,56,57]. Whether these naturally occurring antibodies fail to protect against some strains of *K. pneumoniae*, or if severe comorbidities compromise the effectiveness of natural immunity (or a combination of both) against these microbes, remains unclear. Several published studies have demonstrated the ability of anti-CPS antibodies to confer protection against *K. pneumoniae* in animal models of infection. For example, Cryz et al. demonstrated that rabbit anti-CPS antibodies protect against *K. pneumoniae* sepsis in a murine burn model of infection [58]. In subsequent studies, the authors demonstrated that anti-CPS IgG isolated from human volunteers protects mice against *K. pneumoniae* sepsis [59]. Importantly, the study highlighted the safety of the CPS preparation that was used to immunize humans [59]. Held et al. [60] showed that a murine monoclonal antibody raised against a K2 serotype CPS protects rats against experimental *K. pneumoniae* pneumonia [61]. Rats treated with this mAb had decreased bacterial counts in the lungs and a relatively normal lung histology compared to BSA-pretreated control rats. Additionally, the mAb-pretreated rats maintained a stable body weight and had better motor activity compared to control infected animals that did not receive mAb therapy [60]. In a more recent study by Diago-Navarro et al. [62], mAbs raised against CPS2 from selected ST258 strains were protective in a murine pulmonary infection model. Diago-Navarro et al. [62] found that these anti-CPS2 mAbs enhance serum bactericidal activity toward ST258 strains by increasing C3 complement deposition and MAC formation. The mAbs reduced the number of viable bacteria recovered from lungs compared to infected mice treated with either PBS or isotype control antibody [62].

Immunotherapy studies based on passive immunization against *Klebsiella* spp. were also tested in human clinical trials more than 20 years ago. Cryz et al. [63] generated hyperimmune globulin (IVIG) by vaccinating healthy people with multiple serotypes of *K. pneumoniae* CPS and *Pseudomonas aeruginosa* O-polysaccharide conjugates. This IVIG was then tested in patients admitted to the intensive care units among a group of 16 hospitals [64]. The observed positive outcomes for prevention and moderation of *Klebsiella* infections were not statistically significant and the trial was stopped [64].

Previous studies also investigated passive immunization approaches using LPS-specific antibodies for treatment of *K. pneumoniae* infections. Trautmann et al. [65] generated an LPS-specific mAb that had cross-reactivity with LPS from *K. pneumoniae* O1, O2ab, O2ac, O3, O4, O5 and O12 antigen serotypes. The mAb was produced against unencapsulated *K. pneumoniae* strains and had only limited binding to encapsulated *K. pneumoniae* strains—a major caveat to using O-antigen specific mAbs as an immunotherapy [65]. Rukavina et al. [66] reported similar findings with a mAb (Ru-O1) directed against O1, O6, and O8 serotypes. At high doses, Ru-O1 protected mice against sepsis caused by an encapsulated *K. pneumoniae* strain and significantly reduced bacteria load in the lungs, spleens and livers of infected mice [66]. More recently, Pennini et al. [36] demonstrated that human mAbs raised against the O1 and O2 antigen serotypes protect mice against encapsulated *K. pneumoniae* infection *via* opsonophagocytic killing. Interestingly, sub-therapeutic doses of the anti-LPS mAbs potentiate the activity of meropenem against an intermediate meropenem-resistant strain in a pneumonia infection model [36]. Using sub-therapeutic antibiotic doses could limit potential host toxicities associated with high antibiotic doses and help limit development of antibiotic resistance. A recent study by Follador et al. analyzed hundreds of *K. pneumoniae* clinical isolates and found that 83% were serotyped as O1, O2, or O3 [68]. Given the previously reported ability of mAbs to recognize strains with these O antigens [65,67,69], it is reasonable to suggest that passive administration of a few unique mAbs (e.g., 2–3 mAbs) that are cross-reactive with common O antigens could provide protection against the vast majority of O-antigen serotypes that are clinically prevalent.

3.2. Active immunotherapy

Bacterial capsule polysaccharides have been used historically as vaccine target antigens. For example, capsule polysaccharide vacci-

nes that protect against *Streptococcus pneumoniae* infection are currently licensed for use in humans. One of these vaccines (Pneumococcal 13-valent Conjugate Vaccine or Prevnar 13) is a conjugate vaccine (https://www.fda.gov/vaccines-blood-biologics/vaccines/prevnar-13), whereas the other (Pneumococcal Vaccine Polyvalent or Pneumovax 23) is composed of polysaccharide from 23 serotypes of *S. pneumoniae* (https://www.fda.gov/vaccines-blood-biologics/vaccines/pneumovax-23-pneumococcal-vaccine-polyvalent). These vaccines have been in use for many years (e.g., Pneumovax 23 since 1983) and are examples of successful capsule polysaccharide vaccines. Considering the relative high abundance of hospital-associated *K. pneumoniae* infections, many of which are resistant to antibiotics, it is not surprising that *K. pneumoniae* CPS has been tested previously as a vaccine antigen. Cryz et al. [70] immunized rats with a purified CPS prepared from a *K. pneumoniae* K2 strain. Following challenge with the same strain, the authors showed rats produced high levels of anti-CPS antibodies and immunized rats had significantly fewer bacteria in lungs and blood compared to control rats. Most notably, the CPS vaccine protected rats against fatal pneumonia with minimal lung pathological changes [70]. Postal et al. [71] immunized squirrel monkeys with CPS preparations from a *K. pneumoniae* K5 strain and the vaccine induced a strong immune response characterized by high antibody titers. Immunization was sufficient to protect these monkeys against naturally occurring *K. pneumoniae* infections [71]. Cryz et al. developed multi-valent *Klebsiella* CPS vaccines with specificity for up to 24 different K-antigens [72–74]. These CPS vaccine preparations were well tolerated in human volunteers and they elicited CPS-specific antibody responses [59,72,73]. Inasmuch as a high proportion of CR *K. pneumoniae* infections in the U.S. and worldwide are classified as ST258, and primarily produce CPS1 or CPS2 [14,47,48], combining these polysaccharide antigens is a valid active vaccine approach. Pneumovax 23 is a clinically successful example of a polyvalent vaccine that continues to protect people at risk for pneumococcal infections.

The fact that no licensed *Klebsiella* CPS-based vaccines exist may in part highlight the underlying challenges to developing such a vaccine. Unlike protein or glycoprotein antigens, which elicit T-cell dependent antibody responses, polysaccharide antigens induce T-cell independent antibody responses [75]. T-cell independent antibody responses have traditionally been considered as relatively short-lived because of limited immunological memory [76]. This could potentially be a problem for development of a vaccine based on the *K. pneumoniae* CPS. However, Musher et al. [77] found that antibodies elicited by the 23-valent pneumococcal polysaccharide vaccine last for at least 6 years and antibody levels can be increased by revaccination. Other studies, mainly observational studies, suggest that revaccination with the pneumococcal conjugate vaccine or 23-valent polysaccharide vaccine after a primary immunization with the conjugate vaccine can cause hyporesponsiveness (reviewed in [78]). Additional studies are needed to address these confounding issues, which may also exist for *K. pneumoniae* vaccines.

The immunogenicity of polysaccharides is typically enhanced by a protein carrier conjugate, such as a toxoid or inactive toxin molecule (e.g., CRM197). Seeberger et al. [79] recently reported a method for synthesis of a *K. pneumoniae* glycoconjugate that utilizes CRM197 as a protein carrier. The semi-synthetic glycoconjugate vaccine elicited high titers of cross-reactive CPS-specific antibodies in mice and rabbits that lasted for up to 49 days after vaccination—the longest timepoint tested. Consistent with studies using ST258 CPS2 as an immunogen [53], Seeberger et al. [79] found that the CRM197 glycoconjugate induced antibodies in mice and rabbits that promoted opsonophagocytosis *in vitro*.

4. Summary and outlook

K. pneumoniae is a ubiquitous opportunistic pathogen that causes pneumonia, sepsis and urinary tract infections in immunocompromised individuals and those with debilitating conditions. Antibiotics continue to be the only treatment option for *K. pneumoniae* infections, many of which are caused by multidrug resistant strains and are thus difficult to treat [8,17]. The increasing prevalence of infections caused by antibiotic resistant bacteria underscores the need for alternative treatments to antibiotic therapy. In addition to multidrug resistance, *K. pneumoniae* produces LPS and CPS, which in combination typically hinder host serum bactericidal activity and opsonophagocytic killing (Fig. 1). These molecules have been targeted previously for development of immunoprophylactics and immunotherapeutics, and importantly, such approaches do not rely on heavy antibiotic use. Humanized and/or fully human mAbs are used successfully for prevention and treatment of a wide range of diseases or conditions [80,81]. These mAbs are often used for treatment of specific cancers or inflammatory diseases, but the technology has been applied recently to develop mAbs specific for *K. pneumoniae* [36]. Although there is significant potential for vaccine-based immunotherapies, there are noted caveats. For instance, the relative short half-life of antibodies administered *in vivo* for passive immunotherapy is a limitation of this approach. Vector-mediated *in vivo* antibody expression is a relatively recent method that could be developed further to address this caveat [82,83]. For this method, genes that encode well-characterized antibodies are transferred to non-hematopoietic cells, which then endogenously express and secrete these antibodies [82,83]. This approach has been extensively studied in HIV infections as well as in *Bacillus anthracis* and hepatitis C virus infections [82,83].

Our understanding of the mechanism of capsule synthesis and regulation in *K. pneumoniae* is incomplete. Inasmuch CPS is important for the success of *K. pneumoniae* as an opportunistic pathogen, and because it is a vaccine target antigen, it will be critical to identify the signals that stimulate and/or repress CPS synthesis and unravel the detailed network of molecular receptors and effectors of such signals. A significant portion of what is currently known about capsule synthesis in *K. pneumoniae* is based on the understanding from closely related organisms such as *E. coli* and *Salmonella enterica*. Although these pathogens are gram-negative bacteria, it is perceivable that the mechanisms for capsule synthesis may not necessarily be related. Indeed, a recent study of the capsule regulatory network in *K. pneumoniae* NTUH-K2044 and ATCC 43816 backgrounds by Dorman et al. [84] found strain-specific differences in the genes involved in capsule regulation. Notably, a region of genome recombination in ST258 encompasses the genes involved in capsule biosynthesis, adding further to the potential capsule diversity. These observations collectively highlight the need to properly map out CPS synthesis in the major clinical *K. pneumoniae* isolates.

ST258 rarely causes infections in healthy individuals and has limited virulence in animal infection models compared to *K. pneumoniae* strains known to cause community-associated infections. Comorbidities such as cancers and associated therapies and HIV/AIDS that suppress the immune system, invasive surgical procedures, and prolonged hospitalization are some of the high-risk factors for ST258 infections [8,85,86]. It is noteworthy that immunosuppression can be a significant confounding issue for an active immunization approach. Whether such patients would benefit from active vaccination, which may involve a decrease in gut colonization, remains to be elucidated and would require appropriate animal models. A major impediment to the successful development of vaccines against *K. pneumoniae* is the lack of experimental

models that mimic such human comorbidities and susceptibilities. Owing to the lack of such models, the semi-synthetic glycoconjugate vaccine by Seeberger et al. [79] was not evaluated further. Therefore, the development of CPS-based vaccines must be coupled with development of appropriate animal models that adequately represent groups at risk for infections caused by carbapenem-resistant *K. pneumoniae*.

Conflict of interest

The authors declare no conflicts of interest.

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