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Progress towards the development of Klebsiella vaccines

Myeongjin Choi, **Sharon M Tennant**, **Raphael Simon**, **Alan S Cross**

Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, USA

Abstract

Introduction—Klebsiella pneumoniae (KP) are a leading cause of healthcare-associated infections. The dramatic increase in microbial resistance to third-generation cephalosporin and carbapenem “front line” antimicrobial agents and the paucity of new antimicrobials have left clinicians with few therapeutic options and resulted in increased morbidity and mortality. Vaccines may reduce the incidence of infections thereby reducing the necessity for antimicrobials and are not subject to antimicrobial resistance mechanisms.

Areas covered—We review whole cell, subunit, capsular polysaccharide (CPS), O polysaccharide (OPS) and conjugate vaccines against KP infection, as well as alternative KP vaccine platforms.

Expert opinion—Vaccine-induced antibodies to KP CPS have been protective in preclinical studies, but the number of CPS types (>77) makes vaccines against this virulence factor less feasible. Since four OPS serotypes account of ~80% of invasive KP infections and anti-OPS antibodies are also protective in preclinical studies, both OPS-based conjugate and multiple antigen presenting system (MAPS) vaccines are in active development. Vaccines based on other KP virulence factors, such as outer membrane proteins, type 3 fimbriae (MrkA) and siderophores are at earlier stages of development. Novel strategies for the clinical testing of KP vaccines need to be developed.

Keywords

Klebsiella pneumoniae; antimicrobial resistance; vaccine; conjugate vaccine; O polysaccharide; capsular polysaccharide; MAPS vaccine

1. Introduction

Klebsiella pneumoniae (KP) is a major cause of healthcare-associated infections (HAI) and an important cause of morbidity and mortality. KP was first described by Carl Friedlander in 1882 (and often known as Friedlander’s bacterium). It is a ubiquitous organism found in animals, plants and humans. KP is a leading cause of pneumonia, bacteremia and urinary

Corresponding author: **Alan S. Cross, MD,** Professor of Medicine, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, 685 W. Baltimore, Street, Suite 480, Baltimore, MD 21201, 410-706-5328, Fax: 410-706-6205; cross@som.umaryland.edu.

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tract infections, and often infected burn wounds. Historically KP has been a cause of outbreaks in closed hospital units, particularly in pediatric (neonatal) units. It is also an important cause of infection in spinal injury centers and have caused outbreaks in long-term acute care hospitals (LTACH) and nursing homes.

In recent years a highly virulent KP expressing a hypermucoid K1 capsular polysaccharide (CPS)(positive “string sign”) has infected otherwise healthy individuals worldwide, but predominantly in Southeast Asia and South Africa [1, 2, 3]. It has caused pyogenic liver abscess as well as community-onset pneumonia, meningitis and endophthalmitis.

1.1 Epidemiology of KP and MDR-KP in the USA

A one-day prevalence survey of 11,282 patients in 183 acute care hospitals in the United States revealed that *Klebsiella* spp. and *Staphylococcus aureus* (each at ~10%) were the leading causes of hospital-acquired infections following *C. difficile* [4]. KP was one of the major pathogens as a cause of Gram-negative bacterial (GNB) bloodstream and urinary tract infections (UTI) [4, 5].

1.2 Colonization

KP is part of the normal gastrointestinal flora. It has been found in the stool of over a third of healthy adults not associated with the hospital environment; however, experimentally, one needs to ingest large numbers of KP to establish gastrointestinal (GI) colonization. The carriage rate of KP is higher in the hospital environment, particularly in neonates, than in the community.

KP can be carried asymptomatically in the GI tract and throat, particularly in the elderly and in nursing homes and LTACH, where colonization rates as high as 27% are found. It is also found in the pharynx of hospitalized patients without respiratory disease, but the pharyngeal colonization rate increases dramatically with deteriorating health [6]. Nearly 80% of KP infections are caused by the genetically identical strains of KP that colonize the bowel [7]. Of patients found to have KP on admission to the hospital, 5.2% developed KP infection vs 1.3% who did not [8].

1.3 Antimicrobial resistance (AMR)

AMR is intrinsic to antibiotic use. KP has become increasingly important as the leading etiology of multi-drug resistant (MDR) infections. Well before the current concern over the increasing prevalence of MDR, *Klebsiella* spp. were resistant to multiple antibiotics and acquired resistance during antimicrobial therapy. They also have a central role as a repository and disseminator of MDR genetic determinants to other bacterial species. *Klebsiella* spp. have acquired >400 AMR-associated genes, far more than any other GNB pathogen [9]. Such strains may persist in the environment for years.

In the last two decades there has been a dramatic increase in the resistance of KP to beta-lactam antibiotics, including third generation cephalosporins, commonly employed to treat KP infections. This extended-spectrum beta lactamases (ESBL) resistance, first identified in the US in 1989, forced clinicians to rely on carbapenem antibiotics, but perhaps due to the

pressure of treating ESBL-producing strains with carbapenems, carbapenem-resistant *Enterobacteriaceae* (CRE) emerged in the US in 1996, with carbapenemase-producing KP (KP-C) the most common [10]. In data submitted to the U.S. Centers for Disease Control and Prevention (CDC) databases (National Healthcare Safety Network [NHSN], National Nosocomial Infections Surveillance [NNIS] systems) over the last decade, CRE strains increased from 1.2% to 4.2% of isolates in 2011, with KP becoming the most resistant (1.6% to 10.4%)[11]. KP-C now represents 8% of KP in the United States [12] and up to 28% of KP in the Mid-Atlantic States [13]. Patients infected in the bloodstream with CRE have a higher mortality (20% vs 10%), increased length of stay (LOS) in the hospital and intensive care unit (ICU) and are less likely to be discharged home [14]. Each year KP-C are responsible for 85% of CRE infections in the United States and 520 deaths. Moreover, ESBL-producing KP strains account for ~17,000 nosocomial infections and 1,100 deaths annually [201]. In 2011 additional carbapenemases, such as plasmid-encoded New Delhi metallo-beta-lactamase (NDM-1) and MBL and VIM soon followed. The emergence of MDR KP over the last two decades along with its rapid spread and high mortality led the CDC to declare MDR KP one of three most urgent threats in the US in its landmark 2013 report [201]. MDR KPs necessitate the use of toxic, less effective, “last resort” antibiotics such as polymixin/colistin. Ominously, in 2015, plasmid-mediated resistance to colistin was discovered in an *E. coli* isolate in China conferred by the *mcr-1* gene [15]. Nearly 20% of livestock in China harbor this gene. Aided by air travel, it has now spread globally. Patients from developed countries who travel to less developed countries for less-expensive medical procedures (“medical tourism”) have returned with extensively resistant bacteria in wound infections [202].

2. Microbiology

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic rod and member of the family *Enterobacteriaceae*. *K. pneumoniae* can be differentiated by their capsular polysaccharide (77 K antigens) and lipopolysaccharide (LPS; 8 O antigens) [16]. These two antigens are important virulence factors that can be encountered by the innate immune system [17, 18]. Other important virulence factors include type 1 and type 3 fimbriae which mediate adhesion and biofilm formation and siderophores such as enterobactin and yersiniabactin which acquire iron from the host environment [2].

2.1 O types

Historically 12 KP O antigen serogroups were described, but it was later found that O6, O8, O9 and O11 were identical to other previously described bacteria and were relocated to the genus *Enterobacteriaceae* [19]. Trautmann et al. determined that over 80% of 378 clinical KP isolates from Germany belonged to one of 4 serogroups (O1, O2, O3 and O5) [20]. This finding was corroborated by others [21, 22] and Follador et al.[16] who performed whole genome sequencing on a global collection of more than 500 human and environmental isolates. This study included strains from Australia, Indonesia, Laos, Nepal, Singapore, UK, USA and Vietnam; 67 strains were isolated from blood, 47 strains were from urine and 20 strains were from sputum [16]. LPS O antigens are composed of mannans in O3 and O5

strains and galactans in O1 and O2 strains. Among galactans, D-gal I is capped by the D-gal II polymer in O1 strains, while O2 strains have D-gal I only [23, 24]. Recently, the D-gal III variant of D-gal has been observed as part of the LPS of O1 and O2 of some strains; D-gal III is a branched polysaccharide product resulting from conversion of the D-gal I disaccharide by the *gmlABC* operon [25].

O typing by agglutination with typing antibodies is no longer performed due to the absence of typing sera. However, O types can now be ascertained from whole genome sequence data or by PCR, using a method described by Fang et al. [26] D-galactan III-possessing strains can also be identified by screening O1 and O2 strains for the *gmlABC* locus by PCR [27].

2.2 K types

Early serotyping based on quelling reactions revealed 72 serotypes. There are now more than 77 capsular types of KP [28]. However, only 25 serotypes make up ~70% of clinical isolates [29]. Two capsule types, K1 and K2, are considered to be more virulent than other K types [30, 31]. K1 and K2 strains are more resistant to phagocytosis and intracellular killing [32] and induce less reactive oxygen species [33] than strains of other capsule types.

Just like for O typing, K typing sera are no longer available. Most current methodology to determine K types are molecular based. For example, one popular method involves amplifying and sequencing the *wzi* gene which encodes an outer membrane protein involved in attachment of the capsule to the cell surface [34]. The *wzi* alleles and K types can be identified from the KP sequence typing database at http://bigsdb.web.pasteur.fr. A second common method can identify K1, K2, K5, K20, K54 and K57 by multiplex PCR [35].

2.3 Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) is also commonly used to characterize KP isolates [36]. One particular sequence type (ST), ST258, is associated with carbapenem-resistance and has become dominant worldwide [37]. MLST is performed by amplifying seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*) by PCR and then sequencing the PCR products. Full details are found on the KP MLST website (www.pasteur.fr/mlst) [38]. The MLST database can be used to assign alleles and sequence types (STs).

2.4 Whole genome sequencing

The decreased costs of whole genome sequencing have enabled investigators to study KP diversity at the sequence level [39]. This has been particularly useful for investigation of isolates during outbreaks and to identify the possible source of clinical isolates [40]. A whole genome analysis of hypervirulent KP isolates has also been performed [41].

2.5 Klebsiella Type III major fimbrial protein (MrkA)

The hair-like extensions of KP can be type I or III fimbriae. MrkA is a major protein of the type III fimbriae complex on the surface of KP that is implicated in attachment to host cell and biofilm formation. MrkA forms the central filament of the fimbriae and a minor protein, MrkD, a tip adhesion that caps the fimbrial structure. MrkA has a high degree of sequence conservation and as a KP surface protein, is accessible to antibody. Antibodies against MrkA

can block attachment, reduce biofilm formation *in vitro* and are protective in animal models of infection [42].

2.6 Outer membrane proteins (OMPs) and siderophores

Siderophores, such as aerobactin and enterochelin, are small iron-binding proteins that enable the host to compete for the iron that is necessary for growth. Iron-uptake is dependent on a family of proteins that includes a TonB outer membrane receptor that mediates the transport of iron into the periplasm [42]. The TonB system plays an important role in virulence as TonB mutants are less virulent. Independent of the role of siderophores on bacterial growth, they appear to also modify host immune responses including cytokine secretion, inflammatory gene expression and activation of hypoxia inducible factor-1α (HIF-1α), a master transcription factor that controls vascular permeability and inflammatory gene expression [43]. Hypervirulent KP K1 strains have been associated with increased siderophore activity [10].

3. Role for Vaccines

3.1 Rationale for a vaccine

In 2013 the CDC identified KP-C as an “urgent threat” to healthcare in the United States [201]. Despite the financial and regulatory incentives to facilitate new antibiotic development, during the last 10 years there has been a steady decline in the number of antibiotics submitted for approval to the Food and Drug Administration (FDA), with no new class of antibiotics for GNB introduced in 40 years. Despite their call for an increased effort by pharmaceutical companies to develop new antimicrobials, the World Health Organization noted in a 2017 report that there is a “serious lack” of new antibiotics in development and these are mostly short-term fixes, modifications of existing classes but few innovations. They warned that the world is “headed for a post-antibiotic era…”. With the presence of the *mcr-1* plasmid which confers colistin resistance now detected in clinical isolates in the US, we are faced with the possibility that there will be no effective means of treating serious antibiotic-resistant KP infections.

New antibiotics face the prospect of a relatively short half-life. Bacteria isolated from the “pre-antibiotic” era already had resistance to synthetic antibiotics that did not exist until the 20th century [44, 45]. Further, hundreds of unusual resistance genes were found in AMR bacteria with 11% being in asymptomatic individuals [46]. Thus it is unlikely that the discovery of new antibiotics will circumvent the problem of AMR in the long-term.

The only durable solution to AMR is to prevent infections that necessitate antimicrobial therapy and reduce pathogen transmission. Vaccine-induced antibodies do not drive and are not susceptible to antibiotic resistance mechanisms. Consequently, vaccines are an under-appreciated strategy for addressing AMR.

3.2 Target populations

The development of vaccines for HAIs must be accompanied by strategies for their deployment. Patients undergoing elective surgery or planned interventions that are likely to

put them at risk of developing HAIs (*e.g.* organ transplantation, chemotherapy, immunosuppressive therapies for autoimmune disease) would be prime populations for immunization. Additionally, nearly 50% of patients being discharged from the hospital are likely to be re-admitted in the next 5 years. They can be immunized at discharge with booster doses being given as outpatients. Similarly, patients admitted to long-term acute care facilities and nursing homes are more likely to acquire KP and be a source of dissemination upon admission to an acute care facility. If KP vaccines become as safe and well-tolerated as pneumococcal vaccines, then the vaccine can be considered for all subjects 65 years of age or possibly younger as the likelihood of acquiring a HAI increases with age. Since HAIs often occur late in the course of hospitalization, it may be possible to immunize some patients upon hospital admission. In a pilot study of patients air-evacuated to a shock-trauma facility, all patients immunized with a 24-valent KP CPS vaccine generated an antibody response at 14 days, the first time assessed [47]. A *Pseudomonas* vaccine was given to patients in an ICU. Although the vaccine did not reduce *Pseudomonas*-related mortality, the patients did have a ≥ 4-fold antibody response [48]. Both studies suggest that acutely ill patients do respond to immunization.

4. Review of literature for vaccines developed to date

There is no vaccine for KP currently licensed by the FDA, no KP vaccine clinical studies are reported in ClinicalTrials.gov, and no reports on KP vaccine clinical studies have been published since the mid-1990s. Despite the considerable evidence supporting the protective efficacy of antibodies against KP surface polysaccharides (in 1937 type-specific antisera to KP decreased the mortality of patients with KP pneumonia) [49], with the notable exception of the multivalent *Klebsiella* CPS vaccine that we reported > 2 decades ago, we are unaware of any other *Klebsiella* vaccines or antibody preparations that have been tested in humans.

4.1 Whole cell vaccines

4.1.1 Inactivated vaccines—Although killed/inactivated whole cell vaccines were developed for many human pathogens (e.g. *Bordetella pertussis* vaccine in 1914), the first attempt to develop a killed/inactivated *Klebsiella* vaccine by formalin and acetone was in 1970 [50, 51]. An inactivated vaccine composed of *K. pneumoniae, Escherichia coli, Proteus vulgaris,* and *Enterococcus faecalis* was developed against UTIs in humans. A Phase 2 clinical trial of vaginal mucosal immunization with this vaccine showed 78% of women with recurrent UTIs had no subsequent UTI during the treatment [52]. An orally administered polybacterial immunomodulator (“Dentavax”) consisting of *K. pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Candida albicans* and *Lactobacillus acidophilus* lysates stimulated humoral systemic and mucosal immune responses in humans [53].

4.1.2 Live attenuated vaccines—A live attenuated *Klebsiella* vaccine was first considered in 2008 when a *tonB* deletion mutant elicited IgG which protected mice subsequently challenged with a sub-lethal dose of the wild-type strain [54]. A research team in Iraq reported that KP attenuated using low power laser light could protect mice from lung infection [55]. A crude “ribosomal” *Klebsiella* vaccine was developed at the Institut Pasteur and administered by aerosol or subcutaneous injection to animals and humans [56]. This

approach was abandoned when they found the immunoprotective activity originated from capsular polysaccharide contamination [57].

4.2 Subunit vaccines

4.2.1 Capsular polysaccharides—Capsular polysaccharides (CPS) have long been recognized as a virulence factor for KP. While there are 77 CPS serotypes, clinical trials have been conducted with mono-, hexa- and 24-valent CPS preparations.

Researchers at the Medical Research Council (MRC) Vaccine Research Laboratory in Birmingham, UK studied purified polyvalent-CPS based vaccines in KP mouse challenge models. They found that monovalent and polyvalent vaccines with up to 12 different K antigens could protect mice against *Klebsiella* spp. belonging to 71 different K antigen serogroups [58], but the protection was often short-lived (<14 days). A quadrivalent vaccine showed cross-reaction to many *Klebsiella* capsular types [59].

CPS-based KP vaccines were most intensively studied through the collaborative efforts of investigators at the Swiss Serum and Vaccine Institute and the Walter Reed Army Institute of Research (WRAIR). Encouraged by the success of the 23-valent pneumococcal polysaccharide vaccine and the knowledge that CPS is associated with the virulence of KP and that antibodies to KP CPS were protective in pre-clinical models of infection, this group conducted a seroepidemiology study of >700 bacteremic isolates collected from 13 hospitals in both Europe and North America to determine K serotypes of invasive KP. They determined that a vaccine comprised of the 24 most common K serotypes would cover >60% of clinically relevant KP and an additional 10% of isolates had serotypes that cross-reacted with some of the 24 antigens [29]. Preliminary studies with monovalent (K1) and hexavalent KP CPS vaccines (K2, K3, K10, K21, K30, and K55) found them to be well-tolerated in healthy human volunteers, induced seroconversion in >80% of healthy subjects and the human serum passively protected mice against homologous KP challenge in a burned mouse model [60].

Based on these preliminary studies, these investigators developed a 24-valent CPS vaccine that progressed through multiple clinical trials [61, 62, 63]. These studies also demonstrated safety, immunogenicity and functional (opsonic) activity. The KP vaccine was developed for the purpose of making an intravenous immunoglobulin (IVIG) hyperimmune to both KP and *Pseudomonas* antigens to be given as passive therapy to patients at risk of infection (*i.e.* as prophylaxis) or as treatment to patients actively infected with either of these important clinical pathogens. The 24-valent *Klebsiella* CPS vaccine (Klebvax®) was given to healthy subjects along with a *Pseudomonas* vaccine (Aerugen®) to generate a hyperimmune IVIG (H-IVIG). The KP/PA H-IVIG was tested for its efficacy in preventing serotype-specific KP and PA infections in patients admitted to the ICU in a randomized, double-blinded Veterans Administration Cooperative Study. H-IVIG (725 patients) or human albumen (667 patients) was given to patients upon entry to the ICU. Because of limitations on the supply of H-IVIG, a single infusion of a relatively low dose was administered. While there was some evidence that the IVIG reduced the incidence and severity of vaccine-specific KP infections, the trial was stopped because it was statistically unlikely that the H-IVIG would protect against *Pseudomonas* infections at the dosage being used. In a prospectively identified

cohort of patients that were incubating infection on ICU admission, the H-IVIG-treated patients had a trending, but not significant, reduction in infection [64]. Although this multivalent polysaccharide vaccine was extensively studied in human subjects, it never sought licensure in the United States. Since then considerable advances in vaccine formulation halted further development of this vaccine.

Purified polysaccharides are poorly immunogenic, particularly in neonates, do not induce a memory response, and do not cause somatic hypermutation in B cells that leads to higher affinity antibodies (*i.e.* T-independent antigen). When conjugated to a protein carrier, however, such as tetanus toxoid, a mutant of diphtheria toxin (CRM_{197}) or other proteins, polysaccharides are converted to T-dependent antigens and become more immunogenic. Given the success of polysaccharide-protein conjugate vaccines, it is remarkable that there have been few KP CPS conjugate vaccines developed. Zigterman and colleagues at the University of Utrecht conjugated purified K11 octasaccharade to either keyhole limpet hemocyanin (KLH) or bovine serum albumen (BSA). While the purified CPS induced IgM alone in mice, the conjugate elicited a serum IgG response [65]. More recently, Seeberger and colleagues reported that a synthetic glycoconjugate vaccine comprised of a hexasaccharide from the CPS of a clinically virulent KP strain and conjugated to CRM_{197} was immunogenic and reduced the bacterial burden following infection with the homologous strain; however, it had limited cross-reactivity with other KP CPSs and was not tested for protective efficacy [66].

4.1.2 O (LPS) antigen—To date, development of vaccines based on LPS antigens have not progressed beyond the research stage. While there are currently 8 accepted O serotypes [19], epidemiologic studies suggest that 4 O polysaccharides (OPS) would cover ~80% of clinical isolates world-wide [20]. Antibodies against subcapsular antigens (e.g. LPS) are protective in animal model [22]. Since LPS preparations contain the lipid A moiety responsible for the endotoxic activity, investigators have removed lipid A leaving the core and sugar repeat regions (core and O polysaccharide, or COPS). Purified COPS, like bacterial CPSs, however, are poor immunogens and necessitate novel presentation strategies. Chhibber et al. at Panjab University in India prepared two different KP LPS conjugate vaccines and reported a reduction in lung bacterial load; however ELISA antibody titers were not determined, boost responses were not assessed, and neither was tested in a rodent lethality model [67]. This group also reported that O1 LPS incorporated either into liposomes [68] or sodium alginate microparticles [69] could protect rodents against lobar pneumonia and was less toxic than free LPS, and showed better mucosal immune response than free LPS in mice. Subsequent investigators showed that an O1 LPS vaccine or monoclonal antibodies (mAbs) protected mice against K2:O1 challenges but this protection was short-lived [22].

Recently, we developed a quadrivalent conjugate vaccine comprised of the four most common O serotypes associated with human infections (KP O1, O2, O3 and O5) conjugated to either *Pseudomonas aeruginosa* (PA) flagellin A or B [70]. Since the COPS has the lipid A portion of the LPS chemically removed, it is fully detoxified, thus overcoming limitations of LPS-based vaccines The vaccine induced IgG in rabbits against all 6 antigens that could protect mice against systemic KP infection.

4.1.3 Multiple antigen-presenting system (MAPS) platform—In an effort to reduce reactogenicity and to reproducibly and consistently manufacture vaccines, developers have moved from whole-cell vaccines made from intact microbes that express multiple antigens, including adjuvant-like Toll-like receptor agonists, to subunit or acellular vaccines composed of well-defined, purified antigens. However, these latter vaccines are less immunogenic and usually require adjuvants to boost the immune response. Recently Zhang and colleagues reported on a vaccine platform in which various antigen components, including polysaccharides and proteins, are presented on the same macromolecular complex [71]. This platform has now been used to make a 12-valent vaccine comprised of 4 different KP (O1, O2, O3 and O5) and 8 *Pseudomonas* COPSs and pathogen-relevant proteins, such as MrkA. This vaccine induces robust antibody responses to the COPS of both KP and *Pseudomonas* as well as the proteins. The induced antibodies have functional activity *in vivo* and *in vitro* [72].

4.1.4 Outer membrane proteins—A crude outer membrane protein (OMP) vaccine from an unencapsulated KP strain provided complete protection from bacterial challenge [73]. Since OmpA has been considered an antigen which is involved in host defense mechanism with cytokine production and neutrophil recruitment, it was considered a good vaccine candidate [74]. KP OmpA was first cloned in 1998 [75]. Recombinant OmpK17 and OmpK36 [76] as well as OmpA [77] were used as vaccine candidates against lung infection and sepsis caused by KP in murine models with little [76] to modest [77] protective efficacy. OmpA also has been considered a good carrier protein for polysaccharide haptens that induces CD4+ T cell lymphoproliferative and Th1/Th2 responses as well as primes for cytotoxic T lymphocytes (CTL) epitopes in mice [78]. KP recombinant rP40 OMP has been reported to be an effective carrier protein for other important bacterial polysaccharide vaccine antigens such as a peptide of respiratory syncytial virus [79] or a polysaccharide derived from *Haemophilus* [78]. KP-derived extracellular vesicles, spherical, nanometer-sized proteolipids enriched in OMPs and LPS, induced innate immune responses and both humoral and cellular immunity leading to protection against homologous KP challenge [80].

4.1.5 Fimbriae—While KP express both type 1 and 3 fimbriae, most immune interventions against KP fimbriae have been directed against the type 3, also known as MrkA (see 2.5). Immunization with purified MrkA elicits a robust IgG immune response that protected mice against KP sepsis and lung infection [81]. Anti-MrkA antibodies protected mice from lethal KP pneumonia while immunization with purified MrkA proteins reduced bacterial burden [82]. Type 3 fimbriae isolated from a K26 strain provided protection against challenge with the K2 strain, suggesting a high degree of conservation within type 3 fimbriae [42]. Antibody to the MrkD adhesin has also been shown to be protective [83]. *Klebsiella* type 1 and type 3 fimbriae also have been used as carrier proteins for the core lipooligosaccharide (LOS) from *E. coli* [84]. Both conjugate vaccines induced antibodies to the core LOS, but the type 3 conjugate elicited sera that reacted only with type 3 fimbriae while the type 1 conjugate elicited antisera reactive with both types of fimbriae.

4.1.6 Toxins—In 1983 Klipstein reported that administration of a KP heat-stable enterotoxin protected rats against homologous toxin challenge [85]. Nearly two decades

later, toxoids from purified *Klebsiella* cytotoxin (KCT)-1 elicited complete protection in rodent models against a homologous KP strain, as well as an unrelated KP strain [86] while KCTs II and III were protective only against the homologous KP strain. They also found good passive protection with high titered anti-toxin IgG in baby rabbits born from an immunized mother.

4.1.7 Additional antigens—Various attempts have been made to identify new potential antigen candidates by either *in silico* methods or by genomic screening, using convalescent sera against KP strains. Lundberg *et al* identified 169 proteins by genomic screening, none of which were Omps or fimbriae. Of the 24 candidates selected for immunogenicity and protection studies in mice, 8 elicited varying degrees of protection and depended on the adjuvant used, and these 8 proteins were conserved among ~85% of strains [87]. Lai and colleagues identified 20 proteins expressed in mice during infection using In Vivo Expression Technology (IVET) [88]. No further studies were performed to assess their potential as vaccine candidates. Kurupati et al identified 20 surface proteins that were highly immunogenic and included various OMPs (OmpA, OmpK36, FepA, OmpK17, OmpW), among other proteins. Their preparation of DNA vaccines expressing OmpA and OmpK36 proteins protected mice, with intradermal vaccination inducing better protection than when these vaccines were given by the intramuscular (IM) route [89]. A KP siderophore receptor protein veterinary vaccine (Kleb-SRP) reduced the incidence of KP bovine mastitis and increased milk production when administered before calving [90].

5. Passive immunotherapy

Since infections with MDR KP are associated with high mortality and patients not previously immunized with a putative KP vaccine will require time to develop an antibody response, there has been considerable interest in developing mAb to treat acute KP infections. mAbs against specific CPS and O antigens of KP have been developed, but presumably such antibodies would either have to be part of a therapeutic mAb “cocktail” or rely on the results of a rapid KP typing system which is not presently available [91, 92]. Mandine *et al*. developed protective mAbs against the core-lipid A fraction of KP O1 [93]. Other investigators also identified highly conserved epitopes in the KP LPS core such that a single mAb could theoretically be used to treat a KP infection of unknown serotype [94, 95]. MedImmune has developed mAbs that target multiple KP O types and MrkA [96, 97]. These antibodies had protective activity in murine models of pneumonia. KP-specific bacteriophages have also been considered for therapy [98].

6. Conclusion

KP are major hospital-acquired pathogens associated with urinary tract infection, pneumonia, bacteremia and surgical site infection. Given the increasing multidrug resistance and the reduced pipeline of new antibiotic development, the prevention of KP infection by vaccination is an attractive strategy.

A 24-valent CPS-based KP vaccine was safe and immunogenic in multiple clinical trials since 1970’s; however, a vaccine based on 4 O polysaccharides would provide similar

coverage of KP clinical isolates and when conjugated to a protein carrier such formulations demonstrated good immunogenicity. Recently, new technologies such as the MAPS platform and *in silico*-based antigen screening have been developed. It is anticipated that one of these strategies for KP vaccine development will make an impact on the high morbidity, mortality, and hospital costs associated with KP infection.

7. Expert Opinion

KP has been a leading cause of HAIs for decades, but with only isolated outbreaks of antimicrobial resistance, usually to aminoglycosides, clinicians had alternative antibiotics to which they turned. Consequently, there was no strong impetus to develop a *Klebsiella* vaccine. This has changed dramatically with the increase of KP resistant to frontline (third generation cephalosporin and carbapenem) antibiotics and now the spread of plasmids mediating resistance to colistin, a “last resort” therapeutic. Ominously, there is a dearth of new antimicrobials in the drug development pipeline, particularly as pharmaceutical firms are eliminating their antibiotic development programs and a fragile supply chain is causing shortages of licensed antibiotics. These considerations have led to the specter of a “post-antibiotic” world and the real possibility that some health care facilities may have to limit their cancer treatment, organ transplant and even surgical programs for lack of a means to treat or prevent the inevitable infections that will occur. It is against this backdrop that a renewed interest in *Klebsiella* vaccines and immunotherapy is occurring.

Vaccine targets for KP have been well-identified. Similar to *S. pneumoniae* to which KP CPS have considerable cross-reactions [99], the capsular polysaccharide is a known virulence determinant and the protective efficacy of antibodies directed against this antigen has been well-established; however, the efficacy is only against the homologous CPS. While cross-reactions among many K types have been reported, there is no highly conserved structure among the ~77 K types. A 24-valent CPS vaccine covered nearly 80% of KP clinical isolates and advanced to multiple clinical studies; however, purified polysaccharide vaccines are relatively poorly immunogenic and have been replaced by newer technologies, such as conjugation to protein carriers, formulation in new platforms, such as liposomes or nanoparticles, and the use of adjuvants. Nevertheless, the sheer number of K types and the possibility of geographic differences among the K types encountered make CPS-based KP vaccines less attractive.

The polyvalent, highly cross-absorbed O typing sera developed by Trautmann led to the finding that only a limited number of KP O serotypes accounted for >80% of clinical infections, and this distribution has not changed significantly in the intervening two decades. Thus O-based KP vaccines devoid of lipid A have become feasible. In one format, OPS-protein conjugates do induce robust IgG antibody responses that have functional activity. While tetanus toxoid and CRM_{197} proteins commonly have been used as carriers, our group has shown that pathogen-relevant proteins may similarly be both effective carriers and have the potential benefit of inducing functionally active antibodies against those proteins. As noted in this review, previous investigators have prepared experimental vaccines in which outer membrane proteins and type 3 fimbriae have served as carriers. The OPS-based

Klebsiella MAPS vaccine is currently under development towards the goal of initiating a Phase 1 study in healthy volunteers.

Although many strategies for developing novel vaccine platforms have emerged, several issues will affect the likelihood of seeing an FDA-licensed *Klebsiella* vaccine. Most importantly for this and other vaccines designed to prevent HAIs is the consideration of what type of clinical studies will be required to show efficacy. Although HAIs are an important cause of morbidity and mortality leading to increased hospital LOS and expense, at any one time they occur in only 4% of the hospitalized population [3]. Consequently, it would be difficult to design an adequately powered study where the end-point is prevention of infection. One alternative is to determine if the vaccine can prevent colonization, the precursor of infection, but few vaccines have been licensed on this basis (the pneumococcal conjugate vaccines can prevent nasal colonization with *S. pneumoniae*, but licensure was based on achieving “protective” serum antibody levels). Alternatively, one may seek as a surrogate marker for vaccine efficacy the achievement of a serum antibody level that correlates with protection in a relevant animal model(s). At this writing it is difficult to predict what studies the regulatory agencies may require for licensure.

A second issue that may impact *Klebsiella* vaccine development is the availability of a common, easily performed typing system. The polyclonal, absorbed anti-O typing sera used to identify the most common KP O serotypes in multiple studies is no longer available. In the absence of available antibodies (either highly specific typing sera or mAbs), current investigators have used genotyping systems or multilocus sequence typing. Leaving technical performance issues aside, the genetic typing might not definitively identify a specific antibody-targetable phenotype (K or O), particularly if there are mutations at a specific locus which might not affect the phenotype. MLST is based on the analysis of genes that encode housekeeping proteins that are not virulence factors and therefore not the basis for vaccine development. Moreover, a given MLST type may include different O phenotypes. The need for rapid typing systems for *Klebsiella* K and O serotypes becomes more urgent as investigators and pharmaceutical companies consider the development of mAbs for treatment of active infections. In the absence of multivalent “cocktails”, mAb immunotherapy will depend on the timely identification of both the bacterial species and the serotype.

In the ensuing 5 years some of the *in silico* or proteomic approaches may identify better vaccine candidates and/or conserved antigens that a highly immunogenic and provide broad protection, such as the two studies that identified epitopes in the *Klebsiella* LPS core [94, 95]. Whether these epitopes can be developed into successful vaccines remains to be established. These efforts must be expanded to include much wider coverage of KP serotypes if they are to be transitioned to a clinically useful KP vaccine.

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Declaration of interest

The authors have submitted a patent application for the MAPS *K. pneumoniae* and *P. aeruginosa* vaccine and for the *K. pneumoniae* O polysaccharides and flagellin conjugated vaccines. The work on the MAPS vaccine was supported by Astellas Pharmaceuticals. M Choi, AS Cross, SM Tennant and R Simon, each receive salary support through grants from the NIH, Department of Defense and research agreements with the Nosocomial Vaccine Corporation. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Article highlights

- *Klebsiella pneumoniae* is a leading cause of healthcare-associated infections.
- In the last two decades, *K. pneumoniae* has become resistant to multiple antimicrobial agents, including carbapenem and extended spectrum beta lactam antibiotics, the drugs of choice for serious, life-threatening infections.
- In the face of a diminishing antibiotic development pipeline, vaccines for the prevention of *Klebsiella* infections will play an increasingly important role.
- A *Klebsiella* capsular polysaccharide (CPS) vaccine had progressed through multiple clinical trials and was used to develop hyperimmune immunoglobulin for intravenous use (H-IVIG), but was not further developed in part because of the large number of clinically-relevant CPS types.
- Vaccines based on the 4 most common *Klebsiella* O polysaccharides (LPSs) have been developed as conjugate and multiantigen presenting system (MAPS) vaccines with good immunogenicity in mice and functional activity both *in vitro* and *in vivo*.
- Additional vaccine antigens (e.g. outer membrane proteins, fimbriae) and new vaccine platforms (e.g. nanoparticles, liposomes) have been considered along with the addition of adjuvants.

Table 1.

Klebsiella Vaccines Past and Present

Type of vaccine	Antigen	Administration[f]	Model	Measurement	*In vivo* protection (Y/N)	Reference
Whole cell vaccines	*K. pneumoniae tonB* deletion mutant	IP	Mouse	Evaluated IgG level	Y	[50]
	Live attenuated *K. pneumoniae*	IN or PO	Mouse	Evaluated cytokines and IgA levels	ND	[51]
	Acetone-dried *K. pneumoniae*	IV	Rabbit	Evaluated antibody level	ND	[46]
Subunit vaccines	*K. pneumoniae* cytotoxin	ID	Mouse and Rabbit	Evaluated IgG level in mice and baby rabbit born from immunized mother	Y	[82]
	Purified type 3 fimbriae of *K. pneumoniae*	IP	Mouse	Evaluated IgG level in mice	Y	[77]
	Recombinant AK36 protein comprised of antigens from OmpA and OmpK36	SC	Mouse	Evaluated IgG, IgM, and IgA levels	Y	[73]
	Eight surface proteins[a]	SC	Mouse	ND[g]	Y	[83]
	Siderophore receptor protein	SC	Cattle	Reduced KP mastitis and increased milk production	ND	[86]
Capsular polysaccharide (CPS) vaccines	12 CPSs[b]	IP	Mouse	ND	Y	[54]
	Kl, K36, K44 and K Cross[c]	IP	Mouse	ND	Y	[55]
	24 CPSs[d]	SC	Human	Evaluated IgG	ND	[57]
	K2, K3, K10, K21, K30, and K55	SC	Human	Evaluated IgG level	Y	[58]
O polysaccharide (OPS) vaccines	*Klebsiella* O1 LPS into liposomes	IM	Rat	ND	Y[h]	[64]
	Klebsiella O1 LPS	IM, IT, or IN	Mouse	Evaluated IgG level	Y[h]	[65]
Conjugate vaccines	Octasaccharide derived from CPS (K11) coupled to bovine serum albumin	IP	Mouse	Evaluated IgG level	ND	[61]
	O1 OPS linked to tetanus toxoid	ID	Rat	Alveolar macrophage activation	Y[h]	[63]
	Hexasaccharide 1 coupled to diphtheria toxin mutant	SC	Mouse and Rabbit	Evaluated IgG, and IgM levels	N	[62]
	K. pneumoniae OPS (O1,O2,O3,O5) linked to *P. aeruginosa*(PA) flagellin protein (FlaA, FlaB)	IM	Mouse	Evaluated IgG level	Y	[66]
MAPS vaccine	Four KP OPS (O1,O2,O3,O5) and MrkA[e]	IM	Mouse	Evaluated IgG level	Y	[68]

Type of vaccine	Antigen	Administration[f]	Model	Measurement	*In vivo* protection (Y/N)	Reference
DNA vaccine	Vector pVAX1 expressing OmpA or OmpK36	ID or IM	Mouse	Evaluated cytokines and IgG levels	N	[85]
Vesicle vaccine	Extracellular vesicles	IP	Mouse	Evaluated cytokines and IgG levels	Y	[76]

[a] Glycerophosphodiester phosphodiesterase (GlpQ), recombination regulator RecX, oxidoreductase (YhiN), nitrite reductase subunit (NirB), hypothetical protein (YfhM), DNA repair protein RecO (RecO), glutamine ABC transporter periplasmic protein (GlnH), and penicillin-binding protein 2 (MrdA)

[b] K1, K2, K3, K15, K20, K35, K36, K44, K50, K63, K70, and K74 antigens.

[c] K Cross refers to cross-reactive CPS.

[d] K2, K3, K5, K9, K10, K15, K16, K17,K18, K21, K22, K25, K28, K30, K35, K43, K52, K53, K55, K60, K61, K62,K63, and K64 antigens.

[e] This vaccine also includes *Pseudomonas* OPS and protein.

[f] Intranasal(IN); oral(PO); subcutaneous(SC); intraperitoneal(IP); Intravenous(IV); sublingual(SL); intradermal(ID); intramuscular(IM); intratracheal(IT)

[g] ND, Not determined.

[h] Reduced organ burden, but did not test survival.