**Klebsiella pneumoniae**: Going on the Offense with a Strong Defense

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**SUMMARY**

*K. pneumoniae* causes a wide range of infections, including pneumonias, urinary tract infections, bacteremias, and liver abscesses. Historically, *K. pneumoniae* has caused serious infection primarily in immunocompromised individuals, but the recent emergence and spread of hypervirulent strains have broadened the number of people susceptible to infections to include those who are healthy and immunosufficient. Furthermore, *K. pneumoniae* strains have become increasingly resistant to antibiotics, rendering infection by these strains very challenging to treat. The emergence of hypervirulent and antibiotic-resistant strains has driven a number of recent studies. Work has described the worldwide spread of one drug-resistant strain and a host defense axis, interleukin-17 (IL-17), that is important for controlling infection. Four factors, capsule, lipopolysaccharide, fimbriae, and siderophores, have been well studied and are important for virulence in at least one infection model. Several other factors have been less well characterized but are also important in at least one infection model. However, there is a significant amount of heterogeneity in *K. pneumoniae* strains, and not every infection plays the same critical role in all virulent *Klebsiella* strains. Recent studies have identified additional *K. pneumoniae* virulence factors and led to more insights about factors important for the growth of this pathogen at a variety of tissue sites. Many of these genes encode proteins that function in metabolism and the regulation of transcription. However, much work is left to be done in characterizing these newly discovered factors, understanding how infections differ between healthy and immunocompromised patients, and identifying attractive bacterial or host targets for treating these infections.

**INTRODUCTION**

*K. pneumoniae* has recently gained notoriety as an infectious agent due to a rise in the number of severe infections and the increasing scarcity of effective treatments. These concerning circumstances have arisen due to the emergence of *K. pneumoniae* strains that have acquired additional genetic traits and become either hypervirulent (HV) or antibiotic resistant. *K. pneumoniae* was first isolated in the late 19th century and was initially known as Friedlander’s bacterium (1, 2). It is a Gram-negative, encapsulated, nonmotile bacterium that resides in the environment, including in soil and surface waters and on medical devices (3, 4). Importantly, *K. pneumoniae* readily colonizes human mucosal surfaces, including the gastrointestinal (GI) tract and oropharynx, where the effects of its colonization appear benign (3–5). From these sites, *K. pneumoniae* strains can gain entry to other tissues and cause severe infections in humans. *K. pneumoniae* is an extremely resilient bacterium whose success as a pathogen seems to follow the model of “the best defense for a pathogen is a good defense” rather than “the best defense for a pathogen is a good offense.” This is exemplified by the ability of these bacteria to evade and survive, rather than actively suppress, many components of the immune system and grow at many sites in hosts. This review focuses on *K. pneumoniae* virulence factors that have been studied.
studied in depth and are important in one or more types of infections as well as on additional K. pneumoniae virulence factors that have been identified in recent work. To understand the roles of these factors in the context of K. pneumoniae infections, we first review the different types of K. pneumoniae strains that are now causing significant disease, the types of diseases caused by these K. pneumoniae strains, and the host factors that K. pneumoniae encounters when establishing an infection.

CLASSICAL, ANTIBIOTIC-RESISTANT, EMERGING, AND HYPERVIRULENT STRAINS

Over the last few decades, there has been a concerning rise in the acquisition of resistance to a wide range of antibiotics by strains derived from “classical” K. pneumoniae. As a consequence of this antibiotic resistance, simple infections such as urinary tract infections (UTIs) have become recalcitrant to treatment, and more serious infections such as pneumonias and bacteremias have become increasingly life-threatening (6, 7). Two major types of antibiotic resistance have been commonly observed in K. pneumoniae. One mechanism involves the expression of extended-spectrum β-lactamases (ESBLs), which render bacteria resistant to cephalosporins and monobactams. The other mechanism of resistance, which is even more troubling, is the expression of carbapenemases by K. pneumoniae, which renders bacteria resistant to almost all available β-lactams, including the carbapenems (8).

The first case of K. pneumoniae expressing a carbapenemase was identified in North Carolina in 1996, and thus, this type of carbapenemase is called KPC (9). Additional carbapenemases, such as MBL, NDM-1, IMP, and VIM, have since been found in K. pneumoniae strains (10). Notably, all of these carbapenemases, including KPC, have been found in other bacteria, and collectively, they contribute to the worldwide occurrence of carbapenem-resistant bacteria (10; see references 11 and 12 for recent reviews). Regardless of the type of carbapenemase that they carry, carbapenem-resistant K. pneumoniae isolates are termed CRE, for carbapenem-resistant Enterobacteriaceae. Due to a lack of available effective treatments, K. pneumoniae infections caused by ESBL-producing and carbapenem-resistant bacteria have significantly higher rates of morbidity and mortality than infections with nonresistant bacteria (13). Work reported by the CDC in 2013 demonstrates the frequency and severity of infections with these strains based on a 2011 survey of 183 hospitals in the United States (13). ESBL-producing strains caused 23% of nosocomial K. pneumoniae infections, equaling 17,000 infections, and resulted in 1,100 deaths. Meanwhile, carbapenem-resistant K. pneumoniae strains caused 11% of nosocomial K. pneumoniae infections, equaling 7,900 infections, and resulted in 520 deaths.

The spread of multidrug-resistant K. pneumoniae strains has been an area of intensive investigation over the past decade (10, 12, 14–16). K. pneumoniae strains are typically classified by their sequence types (STs), which are defined by their nucleotide sequences at 7 loci (mdh, infB, tonB, gapA, phoE, pgi, and rpoB), and closely related STs are called clonal complexes (CCs) (reviewed in reference 14). Remarkably, one KPC-carrying K. pneumoniae strain, of ST258 and CC258, has become distributed worldwide within the past 15 years (reviewed in references 10 and 14). Recent genetic analysis of the ST258 strain indicates that this strain arose from a recombination event between an ST11 strain, which is a closely related strain and a member of CC258, and a distantly related K. pneumoniae strain of ST442 (15). The resulting hybrid, the ST258 strain, has a mostly contiguous 1.1-Mbp portion or 20% of its genome derived from ST442, while the remaining 80% of its genome is homologous to that of ST11 (15). Within this 1.1-Mbp region, two regions have undergone notable changes compared to the reference ST442 strain. First, the ST258 strains carry an integrated conjugative element (ICE) (ICEKp258.2) within the 1.1-Mbp area, suggesting that this insertion event occurred before ST258 disseminated worldwide and possibly after the ancestral hybrid strain formed (15). Interestingly, ST11 also carries this ICE, leading to the speculation that this ICE recombined into the ST442 portion of the genome from the parental ST11 strain when ST258 first formed (15). Second, in some ST258 strains, the cps genes encoding the capsule polysaccharide, which are also carried on this 1.1-Mbp area, are unlike those found in ST442 strains (15). In fact, ST258 strains fall into one of two different “clades” depending on the cps gene that they express (17). One clade, clade I, carries cps biosynthetic operons (cps-I) that appear to have been acquired from another distantly related K. pneumoniae strain of ST42, while the cps operon expressed in clade II (cps-II) is similar to those found in ST442 strains (15, 17). These findings suggest that clade I arose from the acquisition of the ST42 cps genes by the clade II ST258 strain. In fact, other K. pneumoniae STs can express a variety of cps genes (18). For example, ST11 strains express at least 3 different cps clusters (15). Analysis of the vast number of sequenced K. pneumoniae strains in the past decade supports the idea that the K. pneumoniae cps locus may frequently undergo genetic changes and that K. pneumoniae strains undergo recombination with other strains, giving rise to additional strains (14, 18). These recent findings regarding the origins of the ST258 strain point toward the idea that K. pneumoniae strains are competent to change rapidly and acquire new traits. Furthermore, the variation observed within ST258 strains and their closely related strains suggests that the reliance of K. pneumoniae on different genetic traits to survive and cause disease likely varies among different strains.

The molecular basis for the rapid and successful worldwide distribution of ST258 remains an area of active investigation but, to date, is still mysterious. Investigators have hypothesized that one or more of the factors encoded on the ICEKp258.2 element found in ST258 and closely related ST strains, such as ST11 strains, may aid in this strain’s ability to persist and spread (19). Specifically, ICEKp258.2 encodes a type IV pilus that could facilitate the strain’s persistence and hardiness by enabling it to adhere to a variety of surfaces and/or by increasing its ability to exchange genetic information (19). A more systematic approach has been used to identify loci that are present in the ST258 strain but absent or present in a nonpandemic strain (ST376) or vice versa (20). In this work, the authors used a “suppressive subtractive hybridization” approach. Genes encoding proteins with a variety of functions that could contribute to the enhanced fitness of strains in ST258 were identified, and these included pilV, components of the pilX operon, and genes encoding efflux proteins, transporters, and enzymes involved in DNA repair, replication, modification, and transfer (20). However, to our knowledge, these specific factors have yet to be tested for their contribution to the fitness of ST258 strains under different conditions.

Likewise, the virulence of KPC-carrying ST258 strains is being tested in a variety of assays and infection models to determine whether wide dissemination correlates with increased virulence. Notably, strains of ST258 are less virulent in mice than another K.
`pneumoniae` strain, ATCC 43816 (ST439), which is frequently used in mouse studies (21, 22). Furthermore, in mouse infections, an ST258 strain was markedly more sensitive to clearance by macrophages than by neutrophils (22), and in cell culture, an ST258 strain was phagocytosed by murine macrophages more efficiently than an ST439 strain (ATCC 43813) (21). The inability of mouse neutrophils to effectively target strains of ST258 is consistent with data from recent studies with human neutrophils, where an ST258 strain was more resistant to killing by human neutrophils than a closely related ST11 strain or a `Staphylococcus aureus` control (23, 24). This overall resistance to killing appears to be derived from inefficient binding to neutrophils (24), because once these strains were phagocytosed, they were rapidly killed (24). There appears to be somewhat ambiguous findings regarding the ability of ST258 strains to be killed by serum. In one study, there was no report of pronounced serum sensitivity of ST258 strains (24). In contrast, an ST258 strain was more sensitive to killing by serum than an ST11 strain (23). Finally, it is important to note that the presence of KPC itself does not appear to correlate strongly with the virulence of ST258 or other `K. pneumoniae` strains (23, 24). Collectively, these findings preliminarily suggest that ST258 strains are not drastically more virulent per se than other `K. pneumoniae` strains, although they appear to have different susceptibilities to various host factors than some other `K. pneumoniae` strains. However, much needs to be done to confirm these findings and to understand whether the spread of strains of ST258 is due to their ability to persist better in the environment and/or in humans.

The worldwide dissemination of ST258 has resulted in the distribution of its associated KPC across a number of different countries and continents (10, 16). The dissemination of and mechanisms of resistance by ESBLs and carbapenemases in `K. pneumoniae` and other bacteria are not the focus of this review, so we refer readers to the other excellent reviews (11, 25). Here, however, we briefly summarize some of the major mechanisms of drug resistance found in `K. pneumoniae`. There are four classes of carbapenemases (classes A to D), and KPCs fall into class A. Sixteen variants of class A KPCs (KPC-2 to -17) have been identified, and KPC-2 and KPC-3 are the best-studied variants to date (26). The carbapenemases found in ST258 strains are encoded on Tn4401, a Tn3-based transposon (14). This transposon has been found on a number of different types of plasmids in ST258 and closely related strains, and these plasmids can be transferred to other `K. pneumoniae` strains (14, 27). To date, the spread of these plasmids and resistance cassettes to other types of bacteria has been much less frequent; however, there are several documented cases (28–30), and it is prudent to expect that more cases will be found or that the transposon may hop into a more promiscuous plasmid. In addition to KPCs, `K. pneumoniae` strains can carry other forms of carbapenemases, such as class B metallo-β-lactamases (such as the New Delhi metallo-β-lactamase NDM-1) and class D OXA enzymes (25). The susceptibility of `K. pneumoniae` to carbapenems depends on several factors. It requires the carriage of these carbapenem resistance genes but often also requires the carriage of other β-lactamases and/or mutations in outer membrane proteins (OMPs) that render the bacteria more resistant to β-lactams, particularly in combination with the expression of a carbapenemase (10, 31). ESBLs, which are also found worldwide, can also be caused by a number of β-lactamases, the most common of which are TEM, SHV, CTX-M, and OXA (11, 25).

Based on the recent emergence and worldwide distribution of ST258, it is clear that `K. pneumoniae` strains can acquire DNA and traits that can change their ability to persist in the environment and human populations. In line with these findings are the observations that strains of the same ST can express different cps genes acquired from strains of different STs. Furthermore, several virulence factors that have been identified in other `K. pneumoniae` strains are not expressed in ST258 strains, indicating that a variety of factors can contribute to the persistence and virulence of `K. pneumoniae`. Combined, these observations indicate that `K. pneumoniae` genomes and phenotypes can change rapidly, and therefore, defining strains and strain types is a moving target. Nonetheless, for the purposes of the remainder of this review, we consider `K. pneumoniae` strains that lack excessive capsule (see below) and, therefore, rarely cause disease in otherwise healthy individuals (with the exception of urinary tract infections) as “classical” strains regardless of whether they are multidrug resistant.

Typically, classical `K. pneumoniae` strains cause serious infections, such as pneumonia, bacteremia, or meningitis, when infecting immunocompromised individuals, including people suffering from diabetes or malignancies (32–34). The carriage and expression of drug resistance do not enhance the virulence of `K. pneumoniae` strains despite making them more difficult to treat. However, since the 1980s, strains of `K. pneumoniae` that can cause serious infections in otherwise healthy individuals have also gained traction in the human population. These strains are considered HV compared to classical `K. pneumoniae` strains due to their ability to infect both healthy and immunocompromised populations and because of the increased tendency of these infections to be invasive; i.e., they can establish infection in the liver (35, 36). This additional virulence correlates with the acquisition of a 200- to 220-kb plasmid containing genes that enhance capsule production as well as encode siderophores (37). These and other bacterial factors that contribute to the hypervirulence of these strains are discussed in detail below (38). An overview of the differences between classical and HV `K. pneumoniae` strains can be found in Table 1.

While infections currently caused by HV strains occur primarily in Taiwan and Southeast Asia versus the worldwide occurrence of infections with classical strains, unsurprisingly, there have been reports of the international spread of HV strains, including into North America (38–40). In contrast to the majority of infections caused by classical `K. pneumoniae` strains, many HV `K. pneumoniae` infections originate in the community (41). HV strains are typically associated with pyogenic liver abscesses, although they can also cause pneumonias, lung abscesses, and other types of infections (41, 42). In fact, HV `K. pneumoniae` has become the most commonly isolated pathogen in lung abscesses in Taiwan, with a report approximating `K. pneumoniae` in lung abscesses in 21% of patients from 2000 to 2004, a shift from a previous report documenting cases from 1985 to 1990, where `K. pneumoniae` was associated with lung abscesses in only 4.6% of cases (43, 44). Troublingly, patients infected with these strains frequently become bacteremic, a condition that is correlated with a significantly worse prognosis (44). Of the people infected by HV `K. pneumoniae`, approximately half of those who acquire infections in the community have underlying diabetes, while the other half are young and healthy and have no underlying predisposing factors. Finally, although antibiotic resistance is a phenomenon associated primarily with classical `K. pneumoniae`, there have been reports of HV `K. pneumoniae` strains that carry ESBLs or carbapenemases.
While caused by HV than classical strains. Some diseases are far more likely to arise from secondary spread from a primary infection in the lungs and bladder (51–53). Classical versus HV strains, it is important to recall that HV strains are far more likely to cause very serious infections such as bacteremia, and these are usually pneumonias or UTIs. Classical K. pneumoniae strains compared to 17% of HV K. pneumoniae strains (47).

### INFECTIONS CAUSED BY K. PNEUMONIAE

*K. pneumoniae* is the causative agent of a range of infections, including, but not limited to, pneumonia, sepsis, UTI, bacteremia, meningitis, and pyogenic liver abscesses. Here, we provide a brief overview of some of the most frequent and/or deadly types of infection as a context in which to place the importance of various virulence factors that we discuss. When considering these infections, it is important to recall that HV strains are far more likely to cause community-acquired and systemic infections in otherwise healthy individuals, but for the most part, these strains are geographically limited to Taiwan and Southeast Asia. In contrast, classical strains of *K. pneumoniae* typically cause serious nosocomial infections or UTIs and can be found worldwide (41, 48–50). While there is a broad overlap of the types of infections caused by classical versus HV strains, some diseases are far more likely to be caused by HV than classical strains.

Primary infections caused by classical *K. pneumoniae* strains are usually pneumonias or UTIs. Classical *K. pneumoniae* strains also cause very serious infections such as bacteremia, and these can be either primary bacteremias or secondary bacteremias that arise from secondary spread from a primary infection in the lungs or bladder (51–53). *K. pneumoniae* pneumonias can be split into two broad categories: community-acquired pneumonias (CAPs) and hospital-acquired pneumonias (HAPs). An overview of these two categories of *K. pneumoniae* infection is presented in Table 2. *K. pneumoniae* HAPs are far more prevalent than *K. pneumoniae* CAPs and are discussed first. The precise definition of a HAP varies based on the publication, but HAP is generally defined as pneumonia that presents at least 48 h after admission to a hospital in individuals with no symptoms of pneumonia prior to admission. Overall, HAPs caused by bacteria are some of the most frequent types of nosocomial infections and are the leading cause of mortality among nosocomial infections (50, 54–57). *K. pneumoniae* is the underlying cause of ~11.8% of HAPs (50). *K. pneumoniae* HAP presents similarly to other nosocomial pneumonias, with respiratory symptoms that may include cough and unilateral pulmonary infiltrates and systemic symptoms that include fever and leukocytosis (34). These HAPs occur in both ventilated and nonventilated patients, and *K. pneumoniae* is the causative agent in 8 to 12% and 7% of these cases, respectively (58–60). Worryingly, but not unexpectedly, there is a significantly higher risk of *K. pneumoniae* being multidrug resistant in nosocomial infections than in community-acquired infections because many patients have been treated with antibiotics and are carrying antibiotic-resistant flora (51). While CAPs are fairly common, they are potentially serious infections that can progress rapidly and lead to hospitalization,
intensive care unit (ICU) stays, and high rates of morbidity and mortality (58). *K. pneumoniae* is rarely the underlying cause of CAPs in North America, Europe, and Australia, as it is estimated to be the causative agent in only ~3 to 5% of CAPs in these regions. It is, however, a more common etiological agent of CAPs in Asia and Africa, where *K. pneumoniae* is second only to *Streptococcus pneumoniae* as the underlying agent, causing about 15% of infections (32, 42, 60–62). While both classical and HV strains can cause CAPs, the comparatively increased prevalence of *K. pneumoniae* as the etiological agent of CAPs in Asia and Africa is likely due, at least in part, to the increased prevalence of hypervirulent strains in these areas (42). Significantly, regardless of geography, both types of *K. pneumoniae* strains are overrepresented as the etiological agents of pneumonias in patients with severe CAPs. Reports estimate that *K. pneumoniae* CAPs comprise 22 to 32% of cases requiring admission to the ICU, with mortality rates in these ICU patients ranging from 45 to 72% (61, 63, 64). In Asia, one report cites *K. pneumoniae* CAPs as the most frequent causative agent of CAPs that required mechanical ventilation, and another report estimated a mortality rate of 55% in hospitalized patients (65, 66). Cases of *K. pneumoniae* CAPs usually present with symptoms typical of acute pneumonias, including cough, fever, leukocytosis, and chest pain. These infections can also display the trademark *K. pneumoniae* characteristic of “certain jelly sputum,” which is the production of thick blood-tinged mucus resulting from high levels of inflammation and necrosis in the lungs (67, 68).

Depending on the patient population, classical *K. pneumoniae* strains are the second or third most frequent cause of UTIs behind *Escherichia coli*, which causes the vast majority of UTIs (69–71). Typically, *K. pneumoniae* accounts for 2 to 6% of nosocomial UTIs and 4.3 to 7% of community-acquired UTIs (72, 73). As with most UTIs, UTIs caused by *K. pneumoniae* are thought to arise from seeding of *K. pneumoniae* from the GI tract (74). The symptoms of these infections are similar to those caused by other bacterial pathogens, which include dysuria, increased frequency and urgency of voiding, and hematuria. While UTIs can typically be treated with antibiotics, concern is growing that there are more people carrying ESBL-producing and CRE *K. pneumoniae* strains in their GI tract. Seeding of these strains into the bladder results in UTIs that are resistant to treatment with many antibiotics, resulting in increased morbidity and prolonged treatment/hospital stays (75, 76).

An extremely serious consequence of *K. pneumoniae* pneumonias and UTIs is their subsequent spread into the blood to cause bacteremia (44, 77, 78). Among Gram-negative pathogens, *K. pneumoniae* is second only to *E. coli* as the causative agent of both community-associated and nosocomial bacteremias (79). One report concerning cases of nosocomial *K. pneumoniae* bacteremia found that 50% of them originated from primary infections in the lungs (77). Alarmingly, *K. pneumoniae* bacteremias have a high fatality rate. Mortality rates following *K. pneumoniae* bacteremia ranged from 27.4 to 37% (33, 80–83). The higher risk of mortality due to *K. pneumoniae* bacteremia is associated with a number of patient factors, including patients being admitted to the ICU, being more than 65 years old, having an underlying malignancy, presenting with pneumonia, requiring mechanical ventilation or urinary catheters, or being alcoholics (33, 80–84). Of grave concern to the medical community is the increase in antibiotic resistance that makes treatment of lung and bladder *K. pneumoniae* infections more difficult and extends the length of time that patients carry *K. pneumoniae* at these sites, allowing more opportunities for *K. pneumoniae* to spread to the bloodstream and brain. Prolonged and difficult-to-treat infections could also increase the frequency of the already dire outcome of *K. pneumoniae* bacteremia. Interestingly, there have been reports of the use of fecal microbiota transplantation to replace *K. pneumoniae* in the GI tract (85, 86).

With the emergence of HV *K. pneumoniae* strains in Taiwan, a different spectrum of *K. pneumoniae* diseases has been observed clinically (37, 38). Many of these diseases are quite severe and can occur in otherwise healthy individuals as well as in individuals with underlying diseases. Most notably, HV strains cause primary liver abscesses in patient populations that do not appear to have any underlying liver disease, unlike other pyogenic liver abscesses caused by polymicrobial sources (87, 88). These liver infections are likely initiated from a breach in host defenses in the GI tract that permits intestinal microbiota to seed tissue sites. In turn, liver abscesses can give rise to a number of other secondary infections as a result of hematogenous spread from the liver. For example, community-acquired *K. pneumoniae* meningitis is rare in most parts of the world but is observed as an infection secondary to community-acquired liver abscess in Taiwan (89–91) as well as nosocomial infections in Taiwan (92, 93). HV *K. pneumoniae* infections can also lead to severe skin and soft tissue infections (e.g., cellulitis, necrotizing fasciitis, and myositis), endophthalmitis, and abscesses in a number of other tissues (e.g., neck, lungs, and kidneys) (89–91, 94, 95).

**PATIENT RISK FACTORS**

People with underlying forms of immunodeficiency are at a much greater risk for infections with classical *K. pneumoniae* strains than the general population. Risk factors for the acquisition of *K. pneumoniae* nosocomial bacteremia include malignancy, diabetes, chronic liver disease, solid-organ transplantation, and dialysis (32, 33). Other risk factors for nosocomial infection are treatment with corticosteroids, chemotherapy (which can overlap in malignancy patients), transplantation, or other treatments or conditions resulting in neutropenia (34). For both HAPs and CAPs, the primary route of bacterial lung infection is by aspiration of microbes colonizing the oropharyngeal tract or, less commonly, the GI tract (54, 96). Certain conditions increase the risk of aspiration of microbes, including alcohol intoxication, radiation therapy, and endotracheal intubation (97). Likewise, the chance that one has of developing a CAP caused by *K. pneumoniae* increases greatly if one has some form of defect in the defenses of the respiratory tract, such as a deficiency in mucociliary clearance, or immunosuppression (98). While, historically, CAP caused by *K. pneumoniae* has been an infection associated with chronic alcoholism, chronic obstructive pulmonary disease and diabetes are also risk factors (99–101). Given the alarming rise in the number of diabetics overall in the general population, one can anticipate that the numbers of CAPs caused by *K. pneumoniae* strains will increase accordingly (102). Furthermore, because about half of community-acquired *K. pneumoniae* bacteremias occur in diabetics, and 14% occur in patients with underlying malignancies, the prevalence of *K. pneumoniae* bacteremias is also likely to increase with the concurrent increase in the prevalence of diabetes (32). In addition, it is also important to realize that the appearance of the HV strains has changed the epidemiology of CAPs caused by *K. pneumoniae* in...
Asia and could further change the epidemiology of *K. pneumoniae* CAPs in the rest of the world as these strains become increasingly pandemic (42).

A common feature of diabetic patients, those with malignancies, and alcoholics is suppression of the innate immune system. Studies in rodent *K. pneumoniae* lung infection models have identified prominent immune defects in neutrophil recruitment, phagocytosis, phagocyte production of cytokines, and antigen presentation that are caused by alcohol intoxication. These deficiencies lead to a defect in *K. pneumoniae* clearance and increased morbidity and mortality (103–107). In patients with malignancies, populations of innate immune cells are often reduced due to the side effects of cytotoxic therapies that kill rapidly dividing cells, including immune cells and malignant cells, resulting in conditions such as neutropenia (108). Finally, diabetics have impaired bacterial defenses, including altered chemokine and cytokine production, neutrophil responses, and phagocytic capabilities (109, 110). These defects arise in conjunction with many other alterations in the immune system that are all likely attributable to altered glucose metabolism and oxidative stress.

Other populations at risk for *K. pneumoniae* infection are neonates and the elderly. Neonates, particularly those who are premature or in the ICU, are at risk due to immature immune defenses, a lack of established microbiota, and the relatively high permeability of the mucosa in the GI tract in these populations (79, 111). *K. pneumoniae* is often the causative agent of sepsis in neonates and is the leading cause of neonatal sepsis in certain developing countries (112). The elderly, the fastest growing segment of the population in developed countries, are more susceptible to many infections due to changes in their immune responses over time that make them less efficacious at controlling pathogens (113, 114). The elderly are the patient cohort associated with the highest risk of mortality caused by *K. pneumoniae*, with some estimates suggesting a mortality rate of 30% in these patients following hospitalization due to *K. pneumoniae* CAPs (113, 115). Both HAPs and CAPs in the elderly are predominantly initiated through aspiration of oropharyngeal flora. Based on several studies where the mean patient age was at least 60 years, *K. pneumoniae* was the causative agent of 17.2% of all CAPs and 6.5 to 11.6% of all HAPs observed (115).

Finally, patients who undergo procedures with reused scopes or other medical equipment or with inserted medical devices or implants have an open highway for *K. pneumoniae* entry. In part due to their fimbriae (see below), some *K. pneumoniae* strains are very sticky and attach to medical devices (116–119). One frequent route of acquisition of *K. pneumoniae* pneumonias is through endotracheal intubation, which increases the risk of acquiring *K. pneumoniae* ventilator-associated pneumonia (VAP), a form of nosocomial pneumonia that develops at least 48 h postintubation (11). This procedure most likely increases the risk of pneumonia by impairing respiratory tract clearance mechanisms, providing a substrate for the formation of bacterium-containing biofilms, and/or allowing the accumulation of bacterium-carrying oropharyngeal secretions around the endotracheal tube cuff (120). Insertion of a catheter is another means of initiating *K. pneumoniae* infection, where the implant allows a point of entry into the urinary tract as well as a substrate on which *K. pneumoniae* can form a biofilm (75, 121).

Carriage of *K. pneumoniae* in the normal flora is directly correlated with *K. pneumoniae* infection, indicating that the proximal source of most *K. pneumoniae* infections originates from an individual’s microbiota. For example, *K. pneumoniae* colonization of the GI tract predisposes patients to infection from both classical strains in hospital settings and abscesses caused by HV *K. pneumoniae* strains (122, 123). Similarly, oropharyngeal *K. pneumoniae* colonization is associated with an increased risk of *K. pneumoniae* VAP (124). The frequency of *K. pneumoniae* colonization at these sites depends on whether a person has been in the hospital or has undergone long-term antibiotic treatment. A lengthier hospital stay is associated with increased *K. pneumoniae* carriage, as *K. pneumoniae* is often spread by the hands of health care workers or contaminated medical devices, while antibiotic treatment removes antibiotic-sensitive microbes from the gut microbiota, thus allowing an initially minor *K. pneumoniae* population to significantly expand (123, 125–127). Strikingly, one study found that only 10% of patients who had a *K. pneumoniae* infection during hospitalization were colonized with *K. pneumoniae* prior to admission (127). Furthermore, 45% of patients who became colonized intestinally with *K. pneumoniae* during hospitalization developed a fulminant *K. pneumoniae* infection with a strain of the same serotype (127). In this study, approximately double the number of patients who carried *K. pneumoniae* in their intestine acquired *K. pneumoniae* infection within 3 weeks of admission to the hospital compared to noncarriers. Strikingly, a 4-fold difference in the number of carriers compared to noncarriers acquiring HAP due to *K. pneumoniae* was observed following a hospital stay of more than 3 weeks (127). As previously noted, antibiotic treatment amplifies the risk of *K. pneumoniae* infection, and recent antibiotic use is an additional independent risk factor for colonization by and infections with ESBL- and carbapenemase-producing *K. pneumoniae* (128–131).

It is worth noting that since the vast majority of people with *K. pneumoniae* infections have underlying medical conditions that have complex and multifactorial impacts on innate immune systems, healthy individuals likely have multiple or redundant mechanisms with which to withstand incidental infection with *K. pneumoniae*. Nonetheless, the growing worldwide diabetes epidemic and the aging of the population in many countries increase the number of people susceptible to *K. pneumoniae* infections. Meanwhile, the spread of HV *K. pneumoniae* strains, as well as the likely spread of antibiotic resistance among both classical and HV strains, may contribute to the rise in morbidity and mortality of *K. pneumoniae* infections in both immunocompromised and healthy populations.

**K. pneumoniae** and Host Immune Defenses

In order for *K. pneumoniae* to cause infection, it has to overcome mechanical barriers as well as humoral and cellular innate immune defenses (132). A number of *in vitro* studies and *in vivo* studies in rodent models have helped to delineate the role of these immune defenses in controlling *K. pneumoniae* infection as well as how *K. pneumoniae* evades or protects against these defenses. However, it is also critical to note that the contribution of different humoral defenses and cell types within the immune response to bacterial clearance varies based on the *K. pneumoniae* strain (22, 133–135). Thus, different strains of *K. pneumoniae* may express different factors that permit them to negate specific host factors. In this review, we discuss several well-studied virulence factors that enable *K. pneumoniae* to navigate, negate, and target host immune defenses, but first, we briefly introduce some of the
critical host defenses that restrict *K. pneumoniae* during infection.

Among the first defenses encountered by a pathogen in the respiratory tract is the mechanical mucociliary elevator, which consists of a blanket of mucus in the respiratory tract that traps particles and microbes and then shuttles them up and out using the ciliary lining. Likewise, in the genitourinary tract, the flow of urine is a strong mechanical force, which, in conjunction with the chemical defense of the low pH of urine, removes *K. pneumoniae* and other bacteria and prevents entry into the bladder (136, 137). To colonize the GI tract, bacteria face a number of obstacles, both physical and chemical (137). Peristalsis pushes out nonadherent microbes, while the turnover of epithelial cells removes adherent microbes. Mucus, similarly to the respiratory tract, also prevents microbe binding to the epithelium and helps shuttle out nonadherent bacteria. Meanwhile, bile and digestive enzymes create a chemically harsh environment.

Once *K. pneumoniae* passes these initial mechanical barriers, it must then overcome humoral and cellular innate defenses. Humoral defenses consist of a wide range of antimicrobial factors with many functions, including those that are opsonic, bactericidal, and bacteriostatic (132, 138). An example of a humoral defense is the complement system, which can mediate bacterial killing by several mechanisms. The activation of the complement cascade can lead to the formation of membrane attack complexes, which insert as pores into bacterial surfaces, causing lysis (139). Additionally, the activation of the complement cascade results in the release of proinflammatory mediators and chemoattractants for immune effector cells. Third, complement can bind to pathogens and serve as an opsonin for phagocytosis. There are also three different mechanisms by which the complement cascade can be activated, and the cascade is divided into three pathways, classical, alternative, and lectin pathways, depending on how it becomes activated (140). The classical pathway is activated by binding of complement to antibody-antigen complexes. The alternative pathway is activated following the spontaneous conversion of C3 into C3b that then binds to bacterial surfaces that lack the inhibitory molecule sialic acid. Finally, the lectin pathway is activated following the binding of mannose-binding lectin to mannose resides on bacterial surfaces (140).

Additional humoral defenses used to restrict bacterial infections include defensins, which are bactericidal factors in the lungs that disrupt the bacterial membrane, and transferrin, which is a bacteriostatic factor that sequesters iron, a necessary growth factor that disrupts the bacterial membrane, and transferrin, which is a carrier for iron. Although many defensins (141). As discussed in detail below, *K. pneumoniae* has mechanisms in place that protect against many but not all of these humoral defenses. For example, most *K. pneumoniae* strains appear to be resistant to complement-mediated lysis as well as opsonophagocytosis, as evidenced by *in vitro* experiments and mouse infection models (142–148). In mouse infection models, no significant differences were found in *K. pneumoniae* bacterial loads in the lungs between complement-depleted and control mice during lung infection (142). In contrast, there is much more strain-to-strain variability in the susceptibility of *K. pneumoniae* to antimicrobial peptides and proteins, such as defensins and surfactants, depending upon the specific antimicrobial (143, 149–152). For example, human beta defensin 1 (HBD-1) and HBD-2 are not as efficient at killing *K. pneumoniae* as HBD-3 (149). Interestingly, several studies have reported that ESBL-producing *K. pneumoniae* strains seem to be more susceptible than non-ESBL-producing strains of *K. pneumoniae* to HBDs (149, 151).

Cellular mediators act both synergistically with and independently of the humoral defenses to control *K. pneumoniae* infection. In the lungs, the first innate cellular effectors encountered are resident alveolar macrophages, which have phagocytic capabilities and mediate the amplification and/or resolution of immune responses through the production of chemokines and cytokines. When *K. pneumoniae* establishes infection, neutrophils are recruited to the infected tissues by chemokines and cytokines produced by macrophages during infection. These chemokines include interleukin-8 (IL-8), CXCL1, complement, and leukotriene B4. Importantly, mouse models of lung infection have shown that alveolar macrophages help control *K. pneumoniae* infection by recruiting neutrophils as well as other mechanisms (22, 153).

In general, neutrophils are well-known first-line responders to bacterial infections, have greater phagocytic capacity and killing capacity than alveolar macrophages, and are important for containment and clearance of *K. pneumoniae* infection (154). Interestingly, it has been shown that optimal clearance of *K. pneumoniae* from the lungs during the first 24 h of infection occurs in the presence of neutrophils and/or CCR2+ monocytes, but the contribution of each cell type to the effective containment of *K. pneumoniae* varies based upon the *K. pneumoniae* strain (22). In fact, as mentioned above, the pandemic ST258 strain appears resistant to neutrophils in a mouse model of infection; however, many other *K. pneumoniae* strains are sensitive (22). Once neutrophils are present in infected tissues, they employ the numerous effector functions in their arsenal to combat *K. pneumoniae* infection. Studies using both human neutrophils and mouse models of *K. pneumoniae* infection have noted the neutrophilic use of a number of processes to contain *K. pneumoniae* (22, 155–171). These processes include phagocytosis/opsonophagocytosis, the production of inflammatory cytokines, and the release of antimicrobial compounds and structures, such as reactive oxygen species, serine proteases (e.g., neutrophil elastase), lactoferrin, lipocalin-2, myeloperoxidase, and neutrophil extracellular traps (NETs), to contain the bacteria and mediate clearance (22, 155–171).

Dendritic cells (DCs) are also involved in containing and controlling *K. pneumoniae* lung infections. At this site, their recruitment and maturation occur during *K. pneumoniae* infection in a Toll-like receptor 9 (TLR9)-dependent manner, which may signal to the cell to optimize intracellular killing upon the accumulation of bacteria within these cells (172). In fact, the role of TLR9 during *K. pneumoniae* lung infection may be primarily in the context of DCs.

Studies on downstream TLR adaptors have highlighted the need for functional TLRs during *K. pneumoniae* infection, where myeloid differentiation primary response gene 88 (MyD88), TRIF, and TIRAP are all necessary for proinflammatory cytokine production and controlling the bacterial population in the lungs. As with many bacterial infections, MyD88 plays a more significant role than TRIF (170, 173, 174). Furthermore, the protective role of MyD88 during infection seems to be mediated through expression in both bone-marrow-derived cells and non-bone-marrow-de-
rived cells, while that of TRIF is mediated through expression in bone-marrow-derived cells only (175). Mice with defective TLR4 signaling have higher K. pneumoniae CFU and greater mortality during K. pneumoniae pneumonia, indicating that TLR4 prevents mouse mortality and high bacterial loads (176–178). Signaling through this receptor is necessary for IL-23 and IL-17 production in the lungs during K. pneumoniae infection (179).

IL-17 is an important cytokine for mediating effective immune responses during K. pneumoniae infection in mice (156). This cytokine is produced during K. pneumoniae pneumonia and assists in controlling infection, including through neutrophil recruitment (156). The production of IL-17 in response to K. pneumoniae infection requires IL-23 stimulation, which likely originates from alveolar macrophages and DCs (180). IL-12 also improves the response to infection by amplifying IL-17 expression through gamma interferon (IFN-γ) production (180). Additionally, both IL-23 and IL-17 have roles very early in infection independent of the production of IL-12 and IFN-γ (180). Cell types expressing IL-17a during lung infection include γδ T cells, CD4 T cells, CD8 T cells, and NK T cells, where IL-17a is predominantly produced by γδ T cells, followed by Th17 cells (181, 182). However, work has shown that γδ T cells but, interestingly, not αβ T cells play protective roles during K. pneumoniae pneumonia, suggesting that IL-17 from αβ T cells is not critical for the control of infection (183, 184). During K. pneumoniae lung infection, γδ T cells are necessary for the early upregulation of certain inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and IFN-γ (183). IFN-γ has a varying importance in K. pneumoniae infection depending on the route of infection (185). During infection with the same strain of K. pneumoniae, IFN-γ is important for the control of K. pneumoniae primary infection and dissemination from the lungs; however, it is not required for protection from systemic infection following intravenous injection (185, 186).

IL-22, which is similar to IL-17 in that it is a Th17 cytokine and is produced during K. pneumoniae infection in an IL-23-dependent manner, is also critical for the control of K. pneumoniae infection (187, 188). IL-22 was shown to be important in both pneumonic and intraperitoneal (i.p.) models of infection. Specifically, depletion of IL-22 with a neutralizing antibody led to increased bacterial loads in the lungs and rates of mortality (187), while overexpression or the therapeutic administration of IL-22 ameliorated K. pneumoniae peritonitis based on mouse mortality and K. pneumoniae bacterial loads (188). One function of IL-22, as well as IL-17, is the induction of lipocalin-2 expression in blood. Both Th17 and Th22 cells were initially thought to be the origin of IL-22 during K. pneumoniae infection (184). However, since recent work has shown that αβ T cells are unnecessary for the control of infection (184), while NK cells both promote the survival of mice during K. pneumoniae pneumonia and produce IL-22 (189), it is now thought that NK cells are the major source of IL-22 and are critical for controlling K. pneumoniae infections in lungs. Another innate lymphoid cell type, mucosa-associated invariant T (MAIT) cells, has also been suggested to play a nonredundant role in controlling infection, particularly in the K. pneumoniae peritonitis model (184, 190, 191). In these experiments, mice lacking these MAIT cells had increased mortality, systemic spread, and abnormal cytokine expression following i.p. infection compared to wild-type (WT) mice.

Another facet of the immune response involved in K. pneumoniae control is the inflammasome. Specifically, deletion of NLRP3, NLRC4, and ASC in mice results in increased bacterial loads and mortality in mouse models of K. pneumoniae pneumonia (182, 192). This increased in mortality may be related, at least in part, to the resulting reduction in proinflammatory cytokine production, including IL-1β, leading to fewer classically activated macrophages as well as decreased cell death through pyroptosis (182, 192, 193).

K. PNEUMONIAE VIRULENCE FACTORS

K. pneumoniae employs many strategies to grow and protect itself from the host immune response. Importantly, many K. pneumoniae mutants are cleared from the lungs of mice more rapidly than wild-type K. pneumoniae strains, suggesting that wild-type K. pneumoniae employs various factors to circumvent early host responses (194). To date, there are four major classes of virulence factors that have been well characterized in K. pneumoniae, and they are discussed in detail in this section (Fig. 1). These virulence factors consist of capsule, including the production of hypercapsule in HV strains; lipopolysaccharide (LPS); siderophores; and fimbriae, also known as pili.

Several other factors were recently identified as being important for K. pneumoniae virulence. However, these factors are not yet thoroughly characterized, and much work remains to be done to fully understand their mechanisms of action and clinical significance. These virulence factors include OMPs, porins, efflux pumps, iron transport systems, and genes involved in allantoin metabolism.

The virulence factors characterized for K. pneumoniae play various roles in different types of K. pneumoniae infection and in different strains of K. pneumoniae. In addition, several recent compelling studies are revealing a number of other factors that play critical roles in mammalian infection. Based on these known virulence factors, the modus operandi of K. pneumoniae appears to be defensive rather than offensive in protecting itself against the host immune response. For example, pathogenic Yersinia species use type III secretion systems to inject toxins into attacking immune cells in order to inactivate the phagocytic capability of these cells (195). In contrast, K. pneumoniae appears to evade, rather than actively suppress, phagocytosis by using capsule to make it more difficult for the bacteria to be bound and taken up by phagocytes (196).

**Capsule**

Capsule, a polysaccharide matrix that coats the cell, is necessary for K. pneumoniae virulence and is arguably the most thoroughly studied virulence factor of K. pneumoniae (79, 197) (Fig. 2). Acapsular K. pneumoniae strains are dramatically less virulent than isogenic encapsulated strains in mouse models, based on decreased bacterial loads in the lungs, lower rates of mouse mortality, and an inability of the bacteria to spread systemically (167, 194, 197, 198). Furthermore, HV K. pneumoniae strains produce a hypercapsule, also known as being hypermucoviscous, which consists of a mucoviscous exopolysaccharide bacterial coating that is more robust than that of the typical capsule. This hypercapsule may contribute significantly to the pathogenicity of HV K. pneumoniae (41, 199, 200).

Both classical capsule and HV hypercapsule are made up of strain-specific capsular polysaccharides termed K antigens (i.e., K1 and K2, up through K78) (201). The genes needed for the
yersiniabactin is made by approximately half of classical and almost all HV phores. Of the siderophores, enterobactin is made by almost all strains, and structures, type 1 and type 3 fimbriae, and secrete iron-scavenging sidero-

![Siderophores](image)

FIG 1 Four well-characterized virulence factors in classical and hypervirulent K. pneumoniae (Kp) strains. There are four well-characterized virulence factors for pathogenic K. pneumoniae capsule, LPS, fimbriabes (type 1 and type 3), and siderophores. Capsule is an extracellular polysaccharide matrix that envelops the bacteria. Classical K. pneumoniae strains produce a capsule that can be of any of the serotypes K1 to K78; K1 and K2 are associated with increased patho-
genicity. HV strains make a hypercapsule, which amplifies the production of capsular material, resulting in a relatively larger capsule, and are predomi-
nantly of the K1 serotype, while the remaining strains are of serotype K2. LPS, an integral part of the outer leaflet of the outer membrane, is produced by both classical and HV K. pneumoniae strains and can be of O-antigen serotypes 1 to 9 (O1 to -9). Both types of K. pneumoniae make membrane-bound adhesive structures, type 1 and type 3 fimbriabes, and secrete iron-scavenging sidero-

![Capsule](image)

FIG 2 Role of capsule in K. pneumoniae virulence. A number of different functions for capsule have been delineated for K. pneumoniae virulence. First, capsule prevents phagocytosis and opsonophagocytosis of the bacteria by immune cells. Second, it hinders the bactericidal action of antimicrobial peptides such as human beta defensins 1 to 3 and lactoferrin by binding these molecules distal from the outer membrane. Third, it blocks complement components, such as C3, from interacting with the membrane, thus preventing complement-mediated lysis and opsonization. Finally, it averts the fulminant activa-
tion of the immune response, as measured by decreased reactive oxygen spe-
cies (ROS), IL-8, IL-6, and TNF-α production, by assiting in the activation of a NOD-dependent pathway and shielding LPS from recognition by immune cell receptors.

production of capsule in both classical and HV K. pneumoniae strains are located on a chromosomal operon, cps, where both the organization and the sequence of the genes are conserved com-
pared to E. coli (202, 203). The cps gene cluster harbors a number of genes involved in capsule production, including wzi, wza, wzb, wzc, gnd, wca, cpsB, cpsG, and guf (204, 205). K antigens have been traditionally assigned by using serological methods (201). Recently, however, K-antigen typing is often performed by se-
quencing of the wzi locus (206–210). This locus is present in all capsular types of K. pneumoniae, and different wzi locus sequences are strongly associated with specific K antigens. The wzi gene en-
codes a surface protein involved in capsule attachment to the outer membrane, where the loss of this protein results in essen-
tially acapsular bacteria (211, 212). wzy, also known as orf4, is involved in the polymerization of capsular polysaccharides, while wza, wzc, orf5, and orf6 are involved in their surface assembly (202, 213). Other genes, particularly those in the cps gene cluster, are involved in the production of the capsule polymer, such as cpsB and cpsG, which encode a mannose-1 phosphate guanyltransferase and a phosphomannomutase, respectively (202, 213). Like E. coli, K. pneumoniae produces a variety of capsule types. The diversity in capsule types arises from the glycosyltransferase activities of wbaP, wbaZ, wcaN, wcaJ, and wcaO (203, 213). Furthermore, comparative analysis of clinical isolates of K. pneumoniae has shown a large amount of diversity in the cps gene cluster se-
quence between strains (203) and even within strain types (14, 23). For example, the cps gene cluster of a given strain will contain either, but not both, of the glycosyltransferases wbaP and wcaJ (203).

In HV K. pneumoniae, capsule production can be enhanced above basal levels in a number of different ways. The expression of two plasmid-borne transcriptional regulators, regulator of mu-
coid phenotype A (rmpA) and rmpA2; the expression of the chromoso-
mal copy of rmpA; and the regulation of the capsule synthesis A and B genes (rcsA and rcsB) can all increase capsule production. Capsule synthesis can also be amplified in response to external cues. For example, increased glucose concentrations re-
sult in the upregulation of capsule production though RmpA, while relatively high extracellular iron concentrations result in the downregulation of capsule production. In fact, 55 to 100% of HV K. pneumoniae strains express at least one copy of rmpA or rmpA2, compared to 7 to 20% of non-HV K. pneumoniae strains (47, 214). These genes positively regulate the cps locus at the transcriptional level, resulting in the hypercapsule phenotype (202, 215–217). However, not all of these genes must be upregulated or even present concurrently (214, 216, 218, 219). Hypercapsule production can be triggered in the absence of rmpA or rmpA2 by chromo-
smal mucoviscosity-associated gene A (magA) (41). magA was dis-
covered in 2004 during a search to identify genes necessary for the hypercapsule phenotype in K. pneumoniae strains isolated from invasive liver disease; magA was found in 98% of invasive versus 29% of noninvasive strains (199, 220). Subsequent work determined that magA is actually specific to K1 strains and is a wzy-like polymerase that is necessary for capsule production. In later publications, magA was redesignated wzy_K1 (221–223), while other serotypes contain different alleles of wzy. For example, there is a wzy allele specific to K2 strains that has been suggested to be referred to as K_A (224).

There may be an association between the K antigens produced by the infecting K. pneumoniae strain and the severity of infection, especially in community-acquired infections. Of the 78 serotypes identified thus far, there is an overrepresentation of certain sero-

types, as only 25 serotypes make up over 70% of the strains iso-
lated from clinical samples (225). Based on the most frequently isolated serotypes collected from patients and results from mouse experiments, K1 and K2 strains are generally more virulent than strains of other serotypes (65, 226, 227). K2 strains are the most prevalent type of K. pneumoniae strain isolated clinically, followed by K1 strains. There are several possible reasons for the increased incidence of virulence of K1 and K2 strains relative to other strains. One thought is that strains of the K1 and K2 serotypes may induce a smaller amount of reactive oxygen species release by human neutrophils than other serotypes and thus survive better in tissues (228). In addition, strains of the K1 and K2 serotypes are more resistant to phagocytosis and intracellular killing by alveolar macrophages and neutrophils than other strains, and this phenotype is independent of whether they are hypercapsule producers (133, 134, 229). For K1 and perhaps K2 strains, this reduced uptake may be due in part to the presence of sialic acid on their surfaces, which may mimic sialic acid typically produced by host cells and allow evasion of host immune cells (133, 230, 231). Furthermore, functional studies have shown that K1/K2 strains may be more resistant to opsonophagocytic uptake by macrophages via mannose/lectin receptors than strains of other serotypes (232, 233). Unlike strains of other serotypes, K1 and K2 strains lack the specific mannose residue repeats that are recognized by two host factors: the mannose receptor on macrophages and lung-secreted SP-A (134, 234). Either bacteria can be bound directly by mannose receptors or SP-A can attach to the bacteria and then be bound by mannose receptors; both forms of mannose receptor binding by macrophages can elicit antimicrobial responses. Therefore, the lack of mannose in strains of the K1 and K2 serotypes prevents efficient lectinophagocytosis and subsequent proinflammatory signals that recruit neutrophils and monocytes, among other functions.

One study characterizing HV K. pneumoniae strains isolated from 4 different continents found that these strains were almost exclusively K1 strains (93%), while the remaining minority were K2 strains (235), a finding consistent with results of previous studies (18, 236–241). Interestingly, in one study, 27/28 of the K1 HV K. pneumoniae isolates belonged to the same clonal complex (CC23), while the two K2 HV strains examined were genetically unrelated and arose from two distinct clonal lineages (ST25 and ST86) (235). The CC23 K1 HV K. pneumoniae strains were significantly more closely related to each other than to other K1 strains, indicating that they likely disseminated globally from a distinct variant (235). However, these CC23 K1 HV K. pneumoniae strains were more closely related to the K1 reference strain used in this study than they were to other K. pneumoniae strains (235). It has been suggested that the increased virulence associated with K1 and K2 HV strains may be due to the concurrent carriage of several virulence-associated factors, namely, hypercapsule and the siderophore aerobactin (see below), compared to their presence in other strains (227). For example, in a mouse liver abscess model, K1 and K2 strains that lacked a hypercapsule, rmpA, and aerobactin were avirulent (227). In fact, HV CC23 strains all carried the genes for two siderophores as well as RmpA on a large plasmid as well as a genomic island with several differences from other K. pneumoniae strains (235). On the other hand, some work suggests that the presence of the K1/K2 serotypes themselves, rather than hypercapsule, is responsible for increased virulence, as typical capsule-producing K1/K2 strains are not significantly less virulent than hypercapsule-producing K1/K2 strains (229).

During K. pneumoniae infection, capsule protects against the host immune response through multiple mechanisms, including inhibiting phagocytosis by immune cells, preventing activation of the early immune response, and abrogating lysis by complement and antimicrobial peptides. Capsular K. pneumoniae strains are significantly less likely than acapsular strains to be phagocytosed by innate immune cells in both the presence and absence of opsonins (196). Capsule-mediated prevention of bacterial binding and internationalization by immune cells helps limit early inflammatory signals, resulting in a less robust induction of the immune response (242). Specifically, studies performed with K. pneumoniae-infected lungs found that capsular strains induced lower levels of the proinflammatory cytokines TNF-α and IL-6 and higher levels of the anti-inflammatory cytokine IL-10 than did acapsular strains (198, 243). Also, capsular strains of K. pneumoniae dampen NF-κB through a mechanism dependent on NOD1 activation, which attenuates IL-1β-induced production of IL-8, a multipotent immune response protein important for neutrophil recruitment and activation (244). Capsule is necessary, although not sufficient on its own, to mediate this effect. Consistent with these cytokine responses, more immune cells are recruited to lungs infected with acapsular than capsular strains (198).

Capsule also contributes to resistance against complement, although work shows that K. pneumoniae strains vary in terms of whether capsule or LPS is the main determinant to complement-mediated lysis (2, 145, 148, 199, 245, 246). In the absence of the O side chain, acapsular strains tend to be more commonly bound by C3, possibly due to the exposure of complement activators on the surface of K. pneumoniae, which results in increased opsonophagocytosis and serum killing via the alternative complement pathway (146, 194). In some cases, capsule binds to antimicrobials produced by the host immune response and prevents the interaction of these molecules with the bacterial surface (143, 247). Capsule both blocks the bactericidal action of HBDs and suppresses their production from airway epithelial cells (149). Interestingly, capsule may be upregulated in the presence of these antimicrobial molecules, as studies have found an upregulation of capsule expression in the presence of the antimicrobials lactoferrin and polymyxin B (248).

In general, the hypercapsule phenotype enhances resistance to a variety of humoral defenses, including complement killing, HBD-1 to -3, and other antimicrobial peptides such as human neutrophil protein 1 and lactoferrin (199). Even though capsule is not usually the most important defense against complement in classical strains, HV K. pneumoniae strains are less sensitive to complement killing than classical strains (249). Furthermore, the hypercapsule has been correlated with increased resistance to phagocytosis by human neutrophils and macrophages compared to a number of classical strains (199, 229, 249). Notably, the presence of fucose in the hypercapsule has been implicated in the evasion of the immune response, such as bacterial avoidance of phagocytosis (250, 251). The wcaG gene is involved in this fucose synthesis and, not surprisingly, is associated with K. pneumoniae virulence (250, 251). One study found that this gene was present in ~88% of clinical isolates with a range of serotypes and was found in both HV and classical strains (252). Furthermore, work with an HV K. pneumoniae strain noted that wcaG was necessary for hypercapsule production, but not LPS production, and virulence in a mouse i.p. injection model (250).
O antigen, a core oligosaccharide, and lipid A. These components stream of TLR4, are more susceptible to where mice lacking TLR4 or MyD88, a signaling protein down-
strated in mouse models of infection, as it is an important virulence factor that protects against humoral defenses but also can be a strong immune activa-
tor. The lipid portion of bacterial LPS, lipid A, is well known for being a potent ligand of TLR4, a pattern recognition receptor. The lipid A inserts into the bacterial membrane and is a potentially potent activator of inflammation. K. pneumoniae may modify its lipid A to make it less inflammatory during infection, and lipid A may also protect against the bac-
tericidal action of cationic antimicrobial peptides. O antigen is the outermost subunit of LPS. It has important roles in protecting against complement, in-
cluding preventing C1q binding to bacteria, which inhibits subsequent activation of the complement pathway, as well as binding C3b away from the outer bacterial membrane and, thus, abrogating bacterial lysis by the complement membrane attack complex.

Lipopolysaccharide
LPS, also known as endotoxin, is a major and necessary compo-
ent of the outer leaflet of the cell membrane of all Gram-negative bacteria (Fig. 3). Although there is considerable variation in LPS structures among bacterial species, it is typically comprised of an O antigen, a core oligosaccharide, and lipid A. These components are encoded by genes in the \textit{wb}, \textit{waa}, and \textit{lpx} gene clusters, respect-
ively (253–256). Unlike the 77 different K antigens that have been documented for \textit{K. pneumoniae} capsule, there have been only 9 different O-antigen types identified in \textit{K. pneumoniae} isolates, and O1 is the most common (257).

LPS is both a benefit and a hindrance for \textit{K. pneumoniae} during infection, as it is an important virulence factor that protects against humoral defenses but also can be a strong immune activa-
tor. The lipid portion of bacterial LPS, lipid A, is well known for being a potent ligand of TLR4, a pattern recognition receptor. TLR4 stimulation leads to the production of cytokines and chemokines that help recruit and activate cellular responses, in-
cluding neutrophils and macrophages, which clear \textit{K. pneumoniae} infection and control spread to other tissues. This has been demo-
strated in mouse models of \textit{K. pneumoniae} bacterial infection, where mice lacking TLR4 or MyD88, a signaling protein down-
stream of TLR4, are more susceptible to \textit{K. pneumoniae} pneuno-
nia and systemic spread (170, 178, 258). In these more susceptible mice, there is impaired cytokine and chemokine production and recruitment of neutrophils. Certain \textit{K. pneumoniae} strains may use capsule to partially shield their LPS from detection by TLRs (2). Work suggests that strains with the K1, K10, and K16 antigens can mask their LPS, while other strains, such as those expressing K2 antigens, cannot (2). A similar phenomenon occurs in HV \textit{K. pneumoniae} strains, where the hypercapsule dampens TLR4 sig-
aling (259). Another method of preventing recognition by the immune response employed by some bacteria, such as \textit{Yersinia pestis}, \textit{Helicobacter pylori}, and \textit{Porphyromonas gingivalis}, is modi-
fication of the LPS to a form that is no longer recognizable by certain immune receptors (260–262). Recent evidence suggests that \textit{K. pneumoniae} may do the same (263). \textit{K. pneumoniae} dem-
strates marked plasticity in its lipid A structure and in certain mammalian tissue sites, including the lungs, will switch to a 2-hydroxyacyl modification of its lipid A in a PhoPQ-regulated, LpxO-
dependent manner. In a manner similar to those of other bacteria that have modified LPSs, such as \textit{Yersinia}, this modified lipid A does not activate the inflammatory response to the same degree as the native form of lipid A, effectively increasing the \textit{in vivo} viru-
ulence of \textit{K. pneumoniae} (260, 263). Furthermore, the lipid A por-
tion of \textit{K. pneumoniae} LPS also plays a beneficial role in virulence, as a mutant strain of \textit{K. pneumoniae} with altered lipid A acylation was attenuated in a mouse model of pneumonia. Additionally, both \textit{in vitro} and \textit{in vivo} experiments have shown that lipid A protects against some cationic antimicrobial peptides (152, 263).

\textit{K. pneumoniae} is recognized by and activates the classical, alter-
native, and lectin complement pathways, although there is heter-
ogeneity among which pathways become activated across \textit{K. pneu-
moniae} strains (147, 264, 265). LPS is the primary means of protection against complement (2). Strains that contain a full-
length O antigen, or “smooth LPS,” are resistant to complement-
mediated killing, while those with truncated or absent O chains, or “rough LPS,” are susceptible to complement-mediated killing, even in the presence of capsule (2). Specifically, complement-re-
sistant strains activate the complement cascade but are not sus-
ceptible to killing due to the O-antigen portion of LPS. The O antigen protects against C3 by binding C3b, a complement com-
ponent that is both an opsonin and part of the pore-forming pro-
cess, far away from the bacterial membrane and abrogating pore formation (2, 139, 144, 266). Furthermore, the absence of the O antigen renders the bacteria more susceptible to the binding of C1q to the cell surface, resulting in the activation of the classical pathway (145). In mouse pneumonic models, \textit{K. pneumoniae} lacking O antigen colonized the lungs to levels equal to those of the WT strain early after infection but was incapable of spreading systemically (266). The authors of this study speculate that the lack of O antigen may render \textit{K. pneumoniae} sensitive to comple-
ment-mediated killing in the bloodstream, thus making the strain less virulent.

Supporting this role for LPS in virulence is work studying genes involved in proper LPS production, including \textit{ige}, which encodes a UDP galacturonate 4-epimerase, and \textit{wabG}, which encodes a GaA transferase (267, 268). The \textit{ige} gene is present in the majority of \textit{K. pneumoniae} isolates, including both disease-causing and commensal strains, and in the absence of this gene, \textit{K. pneumoniae} LPS produces rough LPS and is less capable of causing UTI, pneu-
monia, and sepsis than WT strains (267, 269, 270). Meanwhile, \textit{wabG} is likely present in almost all clinical isolates, with the ma-
Type 1 and 3 Fimbriae

Fimbriae represent another class of K. pneumoniae virulence factors and are important mediators of K. pneumoniae virulence in vivo in a few ways. First, type 1 fimbriae amplify lectinophagocytosis of K. pneumoniae by macrophages and neutrophils. Second, the FimH subunit increases binding to immune cells such as mast cells, leading to increased immune cell activation and subsequent recruitment of neutrophils, which likely increases K. pneumoniae clearance.

Type 1 fimbriae are thin, thread-like protrusions on the bacterial cell surface and are expressed in 90% of both clinical and environmental K. pneumoniae isolates as well as almost all members of the Enterobacteriaceae (279, 280) (Fig. 4). The K. pneumoniae type 1 fimbrial gene cluster is homologous to that of Escherichia coli (116). The fimA gene encodes the subunit FimA, which makes up the majority of the structure, while the adhesive properties are imbued by the minor subunit FimH on the tip, which is encoded by fimH (116, 279, 281, 282). Other genes within the cluster include those encoding the minor structural subunits FimF (116), fimC (116, 118, 279, 281, 282), and FimG; fimI, which encodes the fimbrial chaperone; fimD, which encodes an usher protein; and fimL, which encodes an uncharacterized product necessary for type 1 fimbriae.

Type 3 fimbriae are helix-like filaments. In contrast to type 1 fimbriae, the type 3 fimbria-encoding operon is found in and expressed by almost all K. pneumoniae isolates. In contrast to type 1 fimbriae, type 3 fimbriae are “mannose insensitive” and therefore do not bind mannose. While a specific cell surface receptor has not yet been identified for type 3 fimbriae, MrkA subunits, with the MrkD subunit on the tip. Type 3 fimbriae have been found to be necessary for K. pneumoniae biofilm production and binding to medical devices. MrkD has specifically been found to bind extracellular matrix, such as that exposed on damaged tissues and coating indwelling devices, while MrkA binds abiotic surfaces, such as medical devices both prior to insertion into patients and after insertion when coated with host matrix. Type 3 fimbriae have been shown to have a possibly detrimental role, as their presence on K. pneumoniae increases reactive oxygen species (ROS) production by neutrophils.

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fimbriae, they have been shown to bind extracellular matrix proteins such as type IV and V collagens (288).

*K. pneumoniae* utilizes environmental cues to regulate the expression of its type 1 fimbriae. For example, type 1 fimbria genes are expressed in the urinary tract but not in the GI tract or lungs (116, 289). This observation is in line with the fact that *K. pneumoniae* type 1 fimbriae contribute to UTIs (289). In particular, type 1 fimbriae contribute to the invasion of bladder cells by *K. pneumoniae* and to biofilm formation in the bladder during UTI in a mouse model system but are not needed for early colonization (116, 117). Based on the lack of expression of type 1 fimbriae in the GI tract and lungs, it is not surprising that these structures are superfluous for or even possibly detrimental during GI tract colonization by *K. pneumoniae* and during *K. pneumoniae*-induced pneumonia (116, 289). Notably, in a mouse lung model of infection, a *K. pneumoniae* mutant lacking the *fim* gene cluster was capable of not only colonizing the lungs but also disseminating to the spleen and liver at WT levels (289). This suggests that even a small amount of type 1 fimbria expression may be detrimental for survival in these sites. Along these lines, experiments in a mouse model found that a *K. pneumoniae* strain lacking *fimK*, which resulted in the overexpression of type 1 fimbriae, was attenuated during lung infection (283). However, it should be noted that this phenotype could also be due to the associated decrease in capsule production that occurs in a *fimK* mutant (283).

Like their type 1 counterparts, type 3 fimbriae are not needed for GI tract colonization or for virulence in the lung. Type 3 fimbriae can bind to bladder epithelial cells grown in culture, but in mouse model systems, they do not seem to contribute to UTIs (289–291). Moreover, in these organs, types 1 and 3 are not functionally redundant; i.e., a strain with a double knockout of the clusters encoding both type 1 and type 3 fimbriae had virulence equal to that of a WT strain in the lungs (289). This finding was somewhat surprising, as previous studies had found that type 3, but not type 1, fimbriae could mediate binding to tracheal cells, buccal cells, and lung sections in *vitro* via MrkD (292).

Arguably, the most important clinically significant roles for fimbriae may be in biofilm formation and binding to abiotic surfaces, as the ability of *K. pneumoniae* to bind to surfaces such as indwelling catheters or other devices provides it with an ability to seed vulnerable sites and to persist in patients (116, 289). This observation is in line with the fact that *K. pneumoniae* type 1 fimbriae contribute to UTIs (289). In particular, type 1 fimbriae contribute to the invasion of bladder cells by *K. pneumoniae* and to biofilm formation in the bladder during UTI in a mouse model system but are not needed for early colonization (116, 117). Based on the lack of expression of type 1 fimbriae in the GI tract and lungs, it is not surprising that these structures are superfluous for or even possibly detrimental during GI tract colonization by *K. pneumoniae* and during *K. pneumoniae*-induced pneumonia (116, 289). Notably, in a mouse lung model of infection, a *K. pneumoniae* mutant lacking the *fim* gene cluster was capable of not only colonizing the lungs but also disseminating to the spleen and liver at WT levels (289). This suggests that even a small amount of type 1 fimbria expression may be detrimental for survival in these sites. Along these lines, experiments in a mouse model found that a *K. pneumoniae* strain lacking *fimK*, which resulted in the overexpression of type 1 fimbriae, was attenuated during lung infection (283). However, it should be noted that this phenotype could also be due to the associated decrease in capsule production that occurs in a *fimK* mutant (283).

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Type 3 and, possibly, type 1 fimbriae may also contribute to the delivery, entry, and persistence of *K. pneumoniae* in VAPs. Endotracheal tubes, commonly used to ventilate patients, give bacteria access to the lungs, hinder patient mucociliary clearance, damage host tissues upon insertion, and provide a surface for biofilm formation (299, 300). Type 3 fimbriae assist *K. pneumoniae* in the colonization of endotracheal tubes and, thus, lung infection in two ways: first, MrkA binds directly to plastic surfaces, and second, MrkD facilitates binding to collagen or bronchial cell-derived extracellular matrix-coated surfaces (296). This suggests that in VAP, MrkA may mediate the binding of *K. pneumoniae* to endotracheal tubes prior to or during initial insertion. Once these indwelling devices become coated with host-derived substances, such as collagen or other bronchial tissue-derived extracellular matrix proteins that are released during damage to the tissues upon insertion of the tube, MrkD may facilitate the adherence of *K. pneumoniae* to these tubes and/or to the bronchial tissues themselves (301). Overall, this may lead to increased deposition and persistence of *K. pneumoniae* in the lungs of ventilated patients. This idea is supported by work done with a mouse model of catheter-associated UTI, where the presence of a catheter allowed the persistence of *K. pneumoniae* in the bladder for at least 48 h postinfection (hpi). In contrast, clearance of *K. pneumoniae* was observed in the absence of a catheter (302). In this model, colonization and persistence in the bladder and on the catheter were facilitated by type 1 and 3 fimbriae. Therefore, it is plausible that both types of fimbriae participate in increasing the likelihood of encountering *K. pneumoniae* on abiotic and biotic surfaces.

In healthy individuals, the benefits of fimbriae to *K. pneumoniae* conferred by increased adherence to biotic surfaces may be countered, in part, by their interactions with immune cells. Specifically, the expression of fimbriae by *K. pneumoniae* and by other organisms increases their binding to phagocytes (232). One consequence of the binding of bacteria to phagocytic cells is the triggering of phagocytosis, which often leads to bacterial internalization and killing. *K. pneumoniae* type 1 fimbriae, as well as certain *K. pneumoniae* capsule types, can trigger an opsonin-independent form of phagocytosis called lectinophagocytosis (232, 303, 304). Phagocytes that interact with pathogens via lectins include neutrophils and macrophages (233). Work with *E. coli* bacteria, which have homologous type 1 fimbriae, has shown that type 1 fimbriae are recognized by the opsonin-independent lectin-binding integrin site on CR3 (CD11/CD18) (303). However, this has yet to be shown for *K. pneumoniae*. Generally, an increase in the internalization of extracellular bacteria by phagocytes during any infection leads to increased bacterial killing (234). This then initiates downstream inflammatory events that amplify cytokine production and the activation and recruitment of responding immune cells such as neutrophils. For example, *K. pneumoniae* binds to mast cells through FimH and activates these cells, which induces the release of cytokines that amplify neutrophil recruitment during infection (305). Interestingly, type 1 fimbriae were significantly less stimulatory to neutrophils, as measured by the upregulation of the oxidative response, unless they were in the presence of opsonins; this protection may be linked to masking by the capsule (196, 297, 306–308). In some strains, capsule may inhibit type 1 fimbria expression and may sterically preclude its binding to phagocytic and epithelial cells. While the roles for type 3 fimbriae

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Siderophores

Iron is a limited resource that is required by K. pneumoniae and must be acquired from the environment for K. pneumoniae to thrive during infection. This metal is not readily available in the host during infection, primarily because, as part of the nonspecific immune response, the host sequesters it to restrict the growth of a number of possible pathogens (309, 310). Normally, there is little free iron in host plasma, as it is bound by iron transport molecules such as transferrin. Mammalian hosts can further reduce iron levels upon bacterial infection by shifting the binding of iron to lactoferrin, which is an innate defense protein present in bodily fluids (309, 311). Therefore, K. pneumoniae, like many other bacterial pathogens, must employ tactics to acquire iron from the host in order to survive and propagate during mammalian infection.

The predominant tactic used by many pathogens, including K. pneumoniae, to acquire iron is through the secretion of siderophores, which are molecules that possess a higher affinity for iron than host transport proteins do. Siderophores can steal iron from host iron-chelating proteins or scavenge it from the environment (311). K. pneumoniae strains encode several siderophores, and the expression and contribution of each siderophore to virulence vary (Fig. 6). The production of more than one siderophore by K. pneumoniae may be a means of optimizing successful colonization of different tissues and/or avoiding neutralization of one siderophore by the host (311, 312). Several siderophores are expressed in K. pneumoniae, including enterobactin, yersiniabactin, salmochelin, and aerobactin, and the roles of these different siderophore molecules in K. pneumoniae infection are discussed in detail below. The affinity of these siderophores for iron ranges from aerobactin with the lowest to enterobactin with the highest (313, 314). While the expression of the other siderophores is less conserved, enterobactin expression is almost ubiquitous among both classical and HV K. pneumoniae strains and is therefore considered to be the primary iron uptake system utilized by K. pneumoniae (315–318). In K. pneumoniae, the genes that are required for enterobactin biosynthesis are carried on the chromosome in the entABCDEF gene cluster, while the fepABCDG gene cluster encodes the proteins that mediate its transport, with fepA specifically encoding the uptake receptor (319, 320). Importantly, while fepA expression has been shown to be upregulated during K. pneumoniae infection, suggesting an upregulation of enterobactin functionality, enterobactin is neutralized by the host-secreted molecule lipocalin-2 (321, 322). Lipocalin-2 is a multifunctional protein that has several antimicrobial capabilities and is released from many cell types, including neutrophils, during infection. It is expressed basally, but the transcription of this factor is upregulated by the host in response to K. pneumoniae infection in the respiratory tract (323–325). Lipocalin-2 does not kill K. pneumoniae but rather inhibits its growth by eliminating the ability of K. pneumoniae to scavenge iron from the host by binding to and neutralizing some of its secreted siderophores (326). Lipocalin-2 also has proinflammatory functions: an increase in its production by the host leads to a significant increase in neutrophil recruitment to the site of bacterial infection, most likely through the production of IL-8 (327, 328). Enterobactin, in the absence of lipocalin-2, assists in both colonization and dissemination from the lungs (329). However, in the presence of lipocalin-2, K. pneumoniae strains that produce only this siderophore are cleared (329).

Yersiniabactin was originally discovered in the Gram-negative bacterial pathogen Yersinia as part of a Yersinia high-pathogenicity island, but this siderophore has since been identified in other bacteria, including K. pneumoniae (330). The proteins required for yersiniabactin synthesis are encoded by irp genes, and it is predicted that the transporters required for the secretion of this siderophore are encoded by the ybt and fyu genes, and the uptake receptor is encoded by ybtQ, although this remains to be thoroughly characterized in K. pneumoniae (319, 321, 330). Interestingly, yersiniabactin has been observed in only ~18% of clinical but 90% of HV K. pneumoniae clinical isolates (319, 329). However, in conjunction with enterobactin, it is overrepresented in K. pneumoniae isolates from the respiratory tract (329). Notably, yersiniabactin is expressed during lung infection, and its activity is not inhibited by lipocalin-2 in vivo during early lung infection, likely because its structure significantly differs from that of enterobactin (311, 312, 329). This allows K. pneumoniae to grow to high bacterial loads in the lungs during infection (329). While yersiniabactin seems to be impervious to lipocalin-2, it is unable to acquire the iron required for the growth of K. pneumoniae in the presence of the host protein transferrin (329). Thus, strains that express only the yersiniabactin siderophore are not capable of disseminating from the lungs, likely because transferrin, which is concentrated in blood plasma, prevents the growth of K. pneumoniae.
moniae in blood. Therefore, immunocompetent individuals can likely withstand infection by *K. pneumoniae* strains that produce only yersiniabactin (311, 329). Work also suggests that yersiniabactin may play a role in HV *K. pneumoniae* pathogenesis in i.p. mouse infections that serve as a model for liver abscesses but not in a pneumonic model (319, 331).

Salmochelin is a c-glucosylated form of enterobactin (332, 333). This modification is carried out by genes found on either the chromosome or a plasmid within the *iroA* gene cluster, *iroBCD*E (319). Transport of the iron-laden form is mediated by IroN (320, 334). Importantly, this modification prevents the binding of salmochelin by lipocalin-2, thus preventing siderophore neutralization and lipocalin-2-dependent induction of inflammation (332). Therefore, it is not surprising that salmochelin enhances *K. pneumoniae* colonization of the nasopharynx in a lipocalin-2–sufficient host (327). Because the expression of salmochelin allows nasopharyngeal colonization by *K. pneumoniae* in hosts capable of producing lipocalin-2, one can predict that patient populations infected with salmochelin–positive strains may be less immunocompromised on average and that salmochelin–producing strains are more virulent. In line with this prediction, salmochelin is present in only about 2 to 4% of nosocomial *K. pneumoniae* strains but is much more prevalent in HV *K. pneumoniae* strains, with one study reporting its presence in >90% of HV *K. pneumoniae* strains associated with pyogenic liver abscesses (318, 319, 329).

Aerobactin is a citrate-hydroxamate siderophore. It is rarely expressed by classical nosocomial *K. pneumoniae* clinical isolates, as it is found in only about 6% of classical strains, yet is present in 93 to 100% of HV *K. pneumoniae* isolates (227, 315, 318, 335). The presence of aerobactin is always associated with a hypercapsule, although not all hypercapsulated strains possess this siderophore (227, 311, 335). This association is due to the fact that the aerobactin gene cluster, *tuuABCD*, along with the aerobactin transporter *tuuA* are carried on the same virulence plasmid that carries *rmpA*, an enhancer of capsule production (215, 319, 336–338). Interestingly, while aerobactin is found only rarely in classical strains causing lung infection, it may be the predominant siderophore expressed in certain HV *K. pneumoniae* strains causing lung infection (331). HV *K. pneumoniae* strains have an increased ability to acquire iron through amplified siderophore production, and aerobactin makes up the vast majority of the total siderophores produced in at least one HV *K. pneumoniae* strain (49). Furthermore, one study found that aerobactin, but not enterobactin, yersiniabactin, or salmochelin, is needed for successful infection by HV *K. pneumoniae* in pulmonary and subcutaneous mouse infection models (49, 331). In a mouse model of intraperitoneal HV *K. pneumoniae* infection, aerobactin plays a functionally redundant role with yersiniabactin and salmochelin, where only the simultaneous deletion of all three siderophores attenuates the strain in vivo (319).

**OMPs**

Several other factors were recently identified as having roles in *K. pneumoniae* virulence. However, these factors are not yet thoroughly characterized, and much work remains to be done to fully understand their mechanisms of action and clinical significance. These virulence factors include OMPs, porins, efflux pumps, iron transport systems, and genes involved in allantoin metabolism.

Several OMPs have been noted to be important for *K. pneumoniae* virulence, including outer membrane protein A (OmpA), peptidoglycan–associated lipoprotein (Pal), and murein lipoprotein (LppA), which are encoded by genes of the same names (339). OmpA aids in *K. pneumoniae* virulence, at least in part, through protection against the innate immune response. However, studies using OmpA purified from *K. pneumoniae* or, conversely, OmpA in the context of the whole *K. pneumoniae* bacterium have yielded seemingly conflicting results. The use of purified OmpA led to observations that OmpA binds to bronchial epithelial cells, as well as DCs and macrophages, leading to enhanced cytokine production (340–342). This binding of OmpA to DCs and macrophages occurs through the scavenger receptor LOX–1, which then leads to TLR2 signaling (341, 342). Instillation of purified OmpA into lungs induces the upregulation of cytokine and chemokine production, which leads to subsequent transient neutrophil recruitment (340). On the other hand, OmpA in the context of the bacterium may inhibit cytokine production as well as increase bacterial resistance to antimicrobial peptides such as α-defensin (343). For example, a *K. pneumoniae* ompA deletion mutant caused amplified IL-8 production in bronchial epithelial cells *in vitro* and increased TNF-α and IL-6 levels in mouse lungs compared to an isogenic strain expressing OmpA (344). Furthermore, this *ompA* deletion mutant was attenuated in a mouse model of pneumonia. The differences in outcomes in these studies highlight the importance of studying virulence factors in the context of the bacterium and indicate that results may vary between strains due to the presence of other bacterial antigens and virulence factors that modulate inflammation, such as LPS and capsule.

The contribution of the OMPs peptidoglycan–associated lipoprotein (Pal) and murein lipoprotein (LppA) to *K. pneumoniae* virulence has been characterized only minimally. Work with an HV *K. pneumoniae* strain using isogenic deletion mutants of these two genes in a mouse intraperitoneal infection model found that the loss of these genes resulted in lower bacterial fitness (339). *In vitro* experiments with these strains indicate that the mechanisms behind the increased fitness that these proteins incur may include protection against neutrophil phagocytosis and killing by neutrophils and serum components (339). Furthermore, these proteins likely contribute to the integrity and selective impermeability of the cell membrane in an LPS- and capsule-independent manner and along these lines also strengthen *K. pneumoniae* against anionic detergents and certain antibiotics.

**Porins**

OmpK35 and OmpK36 are porins that, interestingly, are often poorly or not expressed in antibiotic-resistant *K. pneumoniae* strains, including ESBL-producing and carbapenem-resistant strains (345–351). Downregulation of these porins appears to provide an advantage for these bacteria in the face of antibiotic selection, where the porins perhaps serve as a channel that allows the entry of antibiotics into the bacteria (352). Restoration of the expression of *ompK35* or *ompK36* in carbapenem-resistant *K. pneumoniae* strains significantly decreased antibiotic resistance, while deletion of *ompK36* from a K2 HV *K. pneumoniae* strain increased resistance to certain antibiotics *in vitro* (351, 353). Furthermore, while the deletion of *ompK35* did not change the susceptibility of *K. pneumoniae* to certain antibiotics, the concurrent deletion of both *ompK35* and *ompK36* led to antibiotic resistance that was higher than that with even the *ompK36* single-deletion mutant (355). However, porin downregulation may come at a fitness cost in terms of virulence (345). In *in vivo* mouse intraperitoneal...
infection model, the presence of OmpK36 increased *K. pneumoniae* virulence in the absence of OmpK35, but a *K. pneumoniae* strain expressing both OmpK35 and OmpK36 was even more virulent (353). Further studies showed that an *ompK36* deletion mutant could not infect the lungs to WT levels in a pneumonic infection model (354). Further studies evaluating a classical *K. pneumoniae* strain with and without *ompK36* showed that mice infected intraperitoneally or intranasally with this deletion mutant experienced significantly less mortality (345, 354). One mechanism by which OmpK36 may contribute to virulence *in vivo* is by preventing phagocytosis, as demonstrated by the increased uptake of an *ompK36* deletion mutant by human neutrophils (345). This is possibly due to a change in the binding of bacteria to neutrophils conferred by the loss of OmpK36.

**Pumps and Transporters**

AcrAB is an efflux pump that has been implicated in both *K. pneumoniae* virulence and resistance to antibiotics (355–357). This contribution to virulence was demonstrated in a mouse model of pneumonic infection, where infection with an *acrB* deletion mutant in a K2-expressing *K. pneumoniae* strain resulted in a decreased bacterial load in the lungs compared to that with the WT strain, demonstrating that AcrB enhances bacterial fitness in the lungs (355). Furthermore, the *acrB* deletion mutant was more sensitive to exposure to human bronchoalveolar lavage fluid and antimicrobial peptides such as HBD-1 and HBD-2, indicating that this protein is needed for protection against antimicrobial humoral components (355). The increased fitness imparted by AcrB is also supported by work in a *Caenorhabditis elegans* infection model (357). In terms of antibiotic resistance, an *acrB* deletion strain of *K. pneumoniae* is more susceptible than the WT to certain antibiotics such as β-lactams (355). Along these lines, expression of AcrR in clinical strains is associated with antibiotic resistance (358). Overall, these AcrAB functions are likely mediated by the export of detrimental host molecules or antibiotics out of the bacterial cell.

Kfu is an ABC iron transport system that is involved in the acquisition of iron by *K. pneumoniae*, and there is a strong association between the expression of this factor and HV *K. pneumoniae* strains (89, 274, 319). The relevance of this gene to virulence *in vivo* was established in a lethal mouse model of peritonitis with an HV *K. pneumoniae* deletion strain, where an isogenic kfu deletion mutant failed to cause mortality (319). In this model, kfu was necessary for the formation of liver and brain abscesses. In humans, kfu is more frequently found in invasive clinical strains, specifically strains that originate from a liver abscess to cause meningitis or endophthalmitis, than in noninvasive strains (359). This highlights the potential importance of Kfu to clinical infection and the importance of iron acquisition to *K. pneumoniae* virulence.

**Allantoin Metabolism**

Metabolism of allantoin is a method by which bacteria can obtain carbon and nitrogen from their environment (360). An operon containing genes involved in allantoin metabolism was identified in a search for *K. pneumoniae* genes whose transcription was up-regulated in HV *K. pneumoniae* strains compared to classical strains (361). Further work *in vitro* uncovered a dependence of HV *K. pneumoniae* on this operon when allantoin was used as the sole nitrogen source under aerobic conditions. HV *K. pneumoniae* likely utilizes this operon for virulence *in vivo*, as deletion of allS, an activator of the operon involved in this process, resulted in an HV *K. pneumoniae* strain with significantly reduced virulence based on the 50% lethal dose (LD₅₀) in an intragastric model of infection (361). Along these lines, the presence of this allantoin operon is enriched in strains associated with liver abscess versus commensal strains (224, 362). For example, one study found that allS was present in 100% of HV *K. pneumoniae* isolates from Taiwan but in 0% of K2 isolates and non-K1/K2 isolates from patient blood or liver samples (227), and another study found an association between the presence of allS and the invasiveness of primary liver abscess strains (199).

**NEW GENETIC SCREENS TO IDENTIFY MORE PUTATIVE K. PNEUMONIAE VIRULENCE FACTORS**

As evidenced by the relatively few well-characterized virulence factors described above, there is much left to be uncovered about which *K. pneumoniae* factors are required during infection. Two general types of studies have been executed to identify factors critical for *K. pneumoniae* infection: those that involve high-throughput screening to identify mutations that render *K. pneumoniae* avirulent in mouse models and those that involve comparative genomics of clinically isolated versus environmental strains. An overview of these screens is presented in Table 3, and a summary of the hits categorized by function is presented in Table 4. Many of these hits were in genes that are involved in LPS, capsule, and fimbriae. Others (metabolism and transcription factors) could potentially feed into the synthesis and/or production of previously identified virulence factors, or these could reflect different aspects required for *K. pneumoniae* to establish or maintain infection in different niches. Therefore, we expect that further study of these factors will provide important and relevant additional insights into the disease sites infected by *K. pneumoniae* as well as the physiology of *K. pneumoniae* at these sites.

To our knowledge, there are five publications to date reporting the use of high-throughput approaches in mouse models of infection that sought to identify *K. pneumoniae* virulence determinants. Specifically, two screens were performed to identify *K. pneumoniae* genes critical for survival in the lung, two were performed for genes required for intestinal colonization (with one of these screens also looking at UTIs), and one screen was performed in a liver abscess model (167, 363–366). The majority of these screens were performed with classical *K. pneumoniae* strains, although one screen by Tu et al. focused on an HV *K. pneumoniae* strain (366). Two other studies used genomic comparisons between *K. pneumoniae* strains, with one study focusing on classical *K. pneumoniae* and the other focusing on HV *K. pneumoniae*, to identify genes of interest and then characterized the roles of specific genes in *K. pneumoniae* in a mouse lung infection model (168, 367). Finally, four large-scale screens, three with classical *K. pneumoniae* and one with HV *K. pneumoniae* strains, examined *K. pneumoniae* growth in *in vitro* assays and then assessed the importance of these genes in virulence in mouse models of infection (368–371). Specifically, in these studies, genes important for *in vitro* biofilm production and genes contributing to the inhibition of *in vitro* NF-κB signaling were identified, followed by interrogation with pneumonic and intestinal colonization models to assess the contribution of these genes to the virulence of *K. pneumoniae*.

The first reported large-scale high-throughput study used to
Klebsiella pneumoniae Infections

identify K. pneumoniae virulence factors established a mouse model of pneumonia using a rifampin-resistant derivative of K. pneumoniae strain ATCC 43816. Infection with this strain results in systemic spread and significant mortality following intranasal infection. The authors of this study evaluated the fitness of 4,800 signature-tagged K. pneumoniae mutants in the lung and the spleen and identified 106 genes as being essential for K. pneumoniae fitness in this model (167). These genes encode products that are involved in a variety of processes, including cellular metabolism and assembly of the outer membrane and capsule, cell surface proteins, regulators of transcription, and transporters, as well as a number of hypothetical proteins with unknown functions. More recently, the largest high-throughput screen to date for K. pneumoniae was reported and identified more than 300 genes necessary for successful lung infection by K. pneumoniae in the first 24 h (365). In this study, a preliminary characterization of 6 of these genes found that they appear to be important for virulence by playing either a protective role against the immune response or a role in the acquisition of resources. Specifically, those authors found that rfaH promoted serum resistance and capsule production, aroE promoted serum resistance and capsule production, arnE promoted serum resistance, ilvC and ilvD were important for branched-chain-amino-acid synthesis, and copA promoted resistance to copper toxicity (365).

Using a different approach to identify putative virulence genes, Lau et al. utilized subtractive hybridization of genes to compare a nonvirulent strain, IA565, to the classical virulent K. pneumoniae strain ATCC 43816 (168). These authors identified nine genes putatively required for K. pneumoniae virulence, iron, dcoB, orf350, kvgS, ybiT, mrr, fimD, sthB, and fimD, that were present only in the virulent strain. Based on homology to characterized genes in other bacteria, their functions include the acquisition of iron (iron), production and conversion of energy (dcoB), regulation of transcription (orf350), signaling (kvgS), transport of substrates through the membrane (ybiT), restriction of endonuclease activity (mrr), and, as discussed above, adhesion (fimD and sthB).

In a similar vein, Lery et al. (367) performed a comparative analysis of the newly sequenced HV K. pneumoniae ATCC strain 43816. These authors identified nine genes necessary for successful lung infection by K. pneumoniae in the first 24 h (365). In this study, a preliminary characterization of 6 of these genes found that they appear to be important for virulence by playing either a protective role against the immune response or a role in the acquisition of resources. Specifically, those authors found that rfaH promoted serum resistance and capsule production, aroE promoted serum resistance, ilvC and ilvD were important for branched-chain-amino-acid synthesis, and copA promoted resistance to copper toxicity (365).

TABLE 3 Overview of screens identifying Klebsiella pneumoniae virulence factors that either were performed in vivo or identified virulence in follow-up in vivo assays

<table>
<thead>
<tr>
<th>Strain(s)</th>
<th>Library size (no. of clones), type or method</th>
<th>Infection model(s)</th>
<th>Tissue(s) studied</th>
<th>No. of hits</th>
<th>Gene(s) of note</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae ATCC 43816 Rif&lt;sup&gt;R&lt;/sup&gt; (KPPR1)</td>
<td>4,800, STM</td>
<td>In vivo screen, intranasal, C57BL/6 mice</td>
<td>Lungs, spleen</td>
<td>106</td>
<td>wecA, wzyE, rfbB</td>
<td>167</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 43816 Rif&lt;sup&gt;R&lt;/sup&gt; (KPPR1)</td>
<td>25,000, Tn mutagenesis</td>
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Abbreviations: NA, not applicable; STM, signature-tagged mutagenesis; Tn, transposon; Rif<sup>R</sup>, rifampin resistant; Sm<sup>R</sup>, streptomycin resistant.
TABLE 4  *K. pneumoniae* genes involved in virulence identified and/or confirmed in *in vivo* mouse models<sup>a</sup>

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(Continued on following page)
protein; a third encoded proteins putatively involved in adhesion and evasion of the immune response; a fourth encoded a number of phase-related proteins that the authors speculated might play a role in immune evasion through an IL-17-related mechanism; and a fifth encoded phospholipase D family proteins (367). Follow-up experiments on one of the phospholipase D genes, pld1, found that it contributes to K. pneumoniae virulence during murine lung infection by putatively altering cardiolipin metabolism, demonstrating a novel role for lipid metabolism in K. pneumoniae virulence (367).

Based upon previously reported observations that K. pneumoniae reduces NF-κB signaling, Tomas et al. used a high-throughput screen to identify K. pneumoniae genes that dampen NF-κB signaling during infection of the human epithelial cell line A549 (368). Using a gain of NF-κB signaling as a readout, 5,320 transposon mutants were screened, and 114 mutants that no longer prevented NF-κB signaling were identified. These mutations were found predominantly in genes related to metabolism and transport but were also found in envelope- and capsule-related genes. The latter finding is consistent with the hypothesis that K. pneumoniae capsule may mask certain bacterial antigens from recognition by the host immune system. The authors of this study performed further characterizations of two of the factors identified in their screen, waa, which is known to be involved in O-antigen synthesis and promotes virulence in other bacteria, and pula, which is a component of a type II secretion system that had not previously been implicated in bacterial virulence. Supporting the relevance of these genes during infection and the overall approach, K. pneumoniae mutants lacking these genes were attenuated in a pneumonic mouse model.

At least two studies have searched for K. pneumoniae biofilm factors that play a role in a pneumonic mouse model (369, 370). In

**TABLE 4 (Continued)**

<table>
<thead>
<tr>
<th>Category and gene</th>
<th>Infection model(s)</th>
<th>Strain</th>
<th>Putative function</th>
<th>Defect(s) in in vivo screen</th>
<th>Defect in 1:1 comparison</th>
<th>Defect in single strain</th>
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* GI, gastrointestinal colonization; NA, not applicable; HTH, helix-turn-helix; CRP, cyclic AMP receptor protein; ORF, open reading frame. A number of other mutants were noted to be attenuated in the screen by Lawlor et al. (367), but no gene name was given, and so these genes were not included. *, Tu et al. (366) do not distinguish whether mutants were confirmed by 1:1 competition or by single-strain infection, so it is not clear under which condition the defect was observed. Only the top 25 genes identified by Bachman et al. (363) were included here due to the large number of hits.
the first study, Lavender et al. screened 2,000 transposon mutants for the loss of the ability to form biofilms on abiotic plastic surfaces and/or human extracellular matrix (369). Four mutants were unable to make biofilm on at least one of the surfaces tested: yciI, yadH, yuhH, and pduA. When studied in an in vivo pneumonic model, only one mutant, yciI, was attenuated for virulence based on mouse lethality, indicating that some of the functions required for biofilm formation are important in a lung model of infection. In the second study, Boddicker et al. screened a transposon library of 1,175 signature-tagged K. pneumoniae clones to identify mutants with defects in biofilm formation on a human extracellular matrix-coated plate in a flowthrough continuous-culture system (370). Interestingly, five of the eight confirmed mutants were attenuated in a pneumonic mouse model. Of these mutants, three genes are homologues of genes involved in transcriptional regulation (luxR, lyyR, and crp), one gene is important for capsule synthesis (orf12), and one gene is important for fimbra production (fimA). The other three mutants defective in biofilm formation retained full lethality in the mouse model. Two of these mutants lack genes encoding homologues of proteins involved in sugar transport (phosphotransferases EIB and EIC), and one mutant lacks a gene predicted to encode a protein of unknown function (YPTB18488). Combined, the results of this study and that by Lavender et al. suggest that biofilm production most likely does not play an essential role during K. pneumoniae pneumonia after instillation of bacteria into the lungs (369, 370). Rather, the virulence defects observed in the pneumonic model with certain mutants with biofilm defects may be due to pleiotropic functions of these genes.

Two different studies have identified genes critical for intestinal colonization by K. pneumoniae using signature-tagged mutagenesis (364, 365). One study used a K. pneumoniae library of 2,200 signature-tagged transposon mutants in a mouse model of intestinal colonization and identified 44 mutants that, while present in the input, were not recovered from the colon after 5 days (364). The genes important for GI tract colonization had functions in the regulation of transcription, adhesion, membrane transport, metabolism, and DNA-related enzymatic activity, and several had unknown functions. A follow-up study confirmed the importance of a urease identified in the screen for colonization of the GI tract (374). The second signature-tagged mutagenesis screen tested the ability of 1,440 K. pneumoniae signature-tagged transposon mutants to colonize the intestine and bladder. Thirteen genes were needed under both conditions, while six were needed only for establishing a bladder infection (365). Of the genes needed for both intestinal colonization and bladder infection, three were required for LPS production (waaL, waaE, and wbbO), and three encoded cell membrane/surface-associated proteins (pshX, ompA, and surA). The other seven genes included tufA, which encodes a translation elongation factor; hupA, which encodes a DNA-folding protein; arcB, which encodes a metabolic regulator; two genes encoding hypothetical proteins; and two genes encoding proteins with unknown functions. Of those genes identified to be uniquely required for UTIs, two genes were involved in GDP 1-fucose synthesis (gmd and fcl), which is thought to be necessary for proper capsule synthesis by some serotypes; one gene, fimB, was involved in type 1 fimbra production; one gene encoded a hypothetical protein; and two genes encoded proteins of unknown functions.

Another study specifically sought to first identify genes necessary for biofilm production and then evaluate the essentiality of these genes during intestinal colonization (371). Wu et al. measured the ability of 2,500 transposon mutants in an HV K. pneumoniae strain to form biofilms on plastic (371). From their initial screen, the authors identified 23 mutants that displayed decreased biofilm formation. These mutations were in four categories of genes: seven genes were involved in cellular processing and signaling, four genes encoded surface molecules, six were involved in carbohydrate transport or metabolism (including treC), and the remaining six encoded proteins with unknown functions. These authors also identified four mutations that resulted in an increase in biofilm formation. These mutations were in a multidrug resistance gene (sugE), a cold shock protein gene (ospC), as well as two genes that had unknown functions. Following the identification of biofilm-promoting genes, characterization of these mutants in an in vivo oral gavage mouse model demonstrated that treC was important for intestinal colonization. Again, as with the studies of biofilm-defective mutants that were tested in pneumonic models, these results suggest that functions required for biofilms are not generally required for infection of tissues.

Finally, using an HV K. pneumoniae strain, a liver abscess mouse model was used by Tu et al. to test the virulence of 2,880 signature-tagged K. pneumoniae transposon mutants with insertions in noncapsular genes (366). In this model, 28 mutants failed to grow in the liver or spleen following oral infection. In a finding similar to those of studies of other tissue sites outlined above, these genes encoded metabolic proteins; cell surface proteins, including type 1 and type 3 fimbriae; transporters; transcriptional regulators; and proteins with unknown functions. One of these genes, ymdF, was further characterized as having a role in resistance to oxidative stress.

CONCLUSIONS AND PERSPECTIVES

K. pneumoniae is a medically important, yet understudied, pathogen. It causes infections at a variety of sites in humans, including the lungs, bladder, liver, brain, and bloodstream. The alarming increase in the prevalence of drug-resistant K. pneumoniae infections that are challenging, if not impossible, to treat has recently raised our awareness of the fact that although K. pneumoniae was isolated over 100 years ago, only a few virulence factors are well understood, and likewise, critical host defenses for K. pneumoniae infections were not a focus of intense study. This has recently changed in response to the increasing interest and concern regarding the widespread antibiotic resistance of K. pneumoniae as well as the appearance and spread of HV K. pneumoniae strains that are serious pathogens in otherwise healthy individuals.

Overall, the discovery of K. pneumoniae virulence determinants using a variety of genetic approaches has opened up many avenues of research to characterize these genes and understand how they function in different host environments and on different abiotic surfaces. However, much work remains to be done to understand K. pneumoniae physiology in tissues, to understand the virulence of newly emerging strains that have become pandemic within the last decade, and to develop methods to combat these drug-resistant or HV K. pneumoniae strains. Of particular interest is whether the genes important for infection at one tissue site also have roles at other tissue sites. For example, certain genes needed for UTIs may not be required for pneumonia, and vice versa, which appears to be the case for type 1 fimbriae (116). Factors that are important in only one or a subset of tissues will teach us about
the nutrient requirements and host defenses that are present and distinct to those tissues but will restrict their usefulness as novel anti-infective targets for *K. pneumoniae* infections.

It is also important to determine whether genes identified in screens that understandably use strains that are more virulent in mice than many human clinical isolates are critical for the majority of infections with classical *K. pneumoniae* strains identified from patients, HV *K. pneumoniae* strains, and multidrug-resistant *K. pneumoniae* strains. Ideally, future investigations will reveal several classes of genes and pathways important across many types of infections and in many virulent strains, such as in the case of LPS (79). Such proteins and pathways would be excellent candidates for novel drug targets that may be effective against all types of *K. pneumoniae* infections, particularly those that are caused by ESBL- or carbapenemase-producing *K. pneumoniae* and/or HV *K. pneumoniae* strains. Furthermore, it is important to remember that the majority of people presenting with *K. pneumoniae* pneumonia and bacteremia are in hospitals or long-term-care facilities and are immunosuppressed in some manner. Many of the recent searches for virulence factors have focused on identifying virulence factors in “normal” mice; however, a subset of these factors may not be required in immunosuppressed environments (363). In fact, different genes may play a role in immunosuppressed patient subsets that have yet to be identified and characterized. Furthermore, differences in *K. pneumoniae* infection based on patient subsets may translate into different, and more optimal, approaches for the treatment of these patients in the clinic. Therapeutics that restore or complement missing components of immunity in these patients may help prevent or combat *K. pneumoniae* infections. Conversely, therapeutics that target a gene product that is not required for infection of immunosuppressed hosts would not be effective.

As mentioned above, about half of patients infected with HV *K. pneumoniae* are not notably immunosuppressed. Although a few studies aimed at determining what makes these HV *K. pneumoniae* strains more virulent than classical *K. pneumoniae* strains have been reported, there is still work to be done to delineate their differences and identify “Achilles’ heels” of these HV *K. pneumoniae* strains. In terms of therapeutics, conserved targets important for both HV and classical *K. pneumoniae* strains would certainly be the most attractive, as the ability of clinicians to treat patients without waiting for strain diagnostics would likely decrease morbidity and mortality.

It is also important to understand how *K. pneumoniae* escapes from or protects itself against the immunological challenges that it faces in primary sites of colonization in the GI tract and oropharyngeal sites in addition to infection sites such as the lungs, liver, blood, and bladder. In order to successfully cause an infection, *K. pneumoniae* has to overcome the immunological defenses that normally contain it. Even in most patients with some form of immunosuppression, it is unlikely that all arms of the immune response against *K. pneumoniae* have been abrogated. Therefore, it is of interest to investigate how *K. pneumoniae* overcomes these remaining defenses to colonize tissues, replicate to high bacterial numbers at the primary site of infection, and then undergo systemic spread.

In summary, the recent emergence of a number of difficult-to-treat *K. pneumoniae* strains and infections is challenging the medical community to evaluate both host and bacterial factors critical during infection. Given the relatively new appreciation of the evolving diversity of clinical *K. pneumoniae* strains, studies should be done using the many applicable infection models, including pneumonia, UTI, liver abscess, and GI tract colonization, and with a variety of different strains in order to best understand this pathogen, because previous studies have found that the virulence factors of *K. pneumoniae* may have a role at only certain sites of infection. Fortunately, recently, more and more studies using high-throughput approaches to identify virulence factors have been reported, and work is being done in a more directed manner to investigate specific virulence factors and innate immune defenses. Nonetheless, we still have an incomplete picture of the interaction of *K. pneumoniae* with different components of the immune response in different tissues and how its virulence factors overcome host defenses and/or enable it to replicate and establish niches. Continued studies on these facets of *K. pneumoniae* biology, physiology, and interactions with host tissues should drive insights into how to combat *K. pneumoniae* infections.

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