AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology and Molecular Biology Reviews

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

**Michelle K. Paczosa,[a] Joan Mecsas[b]**

Graduate Program in Immunology, MERGE-ID Track, Sackler School of Biomedical Sciences,[a] and Department of Molecular Biology and Microbiology,[b] Tufts University School of Medicine, Boston, Massachusetts, USA

SUMMARY ..... 629
INTRODUCTION ..... 629
CLASSICAL, ANTIBIOTIC-RESISTANT, EMERGING, AND HYPERVIRULENT STRAINS ..... 630
INFECTIONS CAUSED BY *K. PNEUMONIAE* ..... 632
PATIENT RISK FACTORS ..... 633
*K. PNEUMONIAE* AND HOST IMMUNE DEFENSES ..... 634
*K. PNEUMONIAE* VIRULENCE FACTORS ..... 636
Capsule ..... 636
Lipopolysaccharide ..... 639
Type 1 and 3 Fimbriae ..... 640
Siderophores ..... 642
OMPs ..... 643
Porins ..... 643
Pumps and Transporters ..... 644
Allantoin Metabolism ..... 644
NEW GENETIC SCREENS TO IDENTIFY MORE PUTATIVE *K. PNEUMONIAE* VIRULENCE FACTORS ..... 644
CONCLUSIONS AND PERSPECTIVES ..... 649
ACKNOWLEDGMENTS ..... 650
REFERENCES ..... 650

## SUMMARY

*Klebsiella 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 factor 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

*Klebsiella 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

**Published** 15 June 2016

**Citation** Paczosa MK, Mecsas J. 2016. *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiol Mol Biol Rev 80:629–661. doi:10.1128/MMBR.00078-15.

Address correspondence to Joan Mecsas, Joan.Mecsas@tufts.edu.

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-1*) that appear to have been acquired from another distantly related *K. pneumoniae* strain of ST42, while the *cps* operon expressed in clade II (*cps-2*) 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 nonepidemic 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 monocytes 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 Tn*4401*, a Tn*3*-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

**TABLE 1** Characteristics of classical and hypervirulent *Klebsiella pneumoniae* strains

| Parameter | Characteristic(s) for strain type | | References |
|---|---|---|---|
| | Classical | Hypervirulent | |
| Common types of infection | Pneumonia, UTI, bacteremia | Pyogenic liver abscess; bacteremia; lung, neck, and kidney abscesses; pneumonia; cellulitis; necrotizing fasciitis; myositis, meningitis; endophthalmitis | 37, 42 |
| Susceptible population(s) | Immunosuppressed (diabetics, patients with malignancies) | Diabetics, healthy people | 32, 34, 35, 43, 44 |
| Capsule type(s) | Capsule serotypes K1–K78 | Hypercapsule serotype K1 (93%) or K2 | 38, 79, 226, 227, 235 |
| Siderophores (% of strains expressing siderophore) | Enterobactin (100), yersiniabactin (17–46), salmochelin (2–4), aerobactin (6) | Enterobactin (100), yersiniabactin (90), salmochelin (>90), aerobactin (93–100) | 227, 318, 319, 321, 329, 335 |
| Geographical concentration | Worldwide | Primarily Taiwan and Southeast Asia | 38–41 |
| Primarily acquired infection type | Nosocomial | Community acquired | 41, 48, 49 |
| Frequency of reports of antibiotic resistance | Frequent (ESBL and carbapenemase producing) | Infrequent | 45, 324, 325 |

(45, 46). One recent study in China found ESBLs in 56% of 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,

**TABLE 2** Characteristics of nosocomial and community-acquired *Klebsiella pneumoniae* infections

| Parameter | Characteristic(s) for infection type | | Reference(s) |
|---|---|---|---|
| | Nosocomial | Community acquired | |
| Types of infection | Pneumonia, UTI, bacteremia | Pyogenic liver abscess, UTI, meningitis | 32, 37, 38, 50, 90, 91, 375 |
| Frequency as etiological agent of pneumonia (%) | 11.8 | 3–5 in North America, Europe, and Australia; 15 in Asia and Africa | 42, 50, 60, 62, 79 |
| Frequency as etiological agent of UTIs (%) | 2–6 | 4.3–7 | 69–73 |
| Common underlying conditions | Diabetes, malignancies | Malignancies, diabetes, chronic obstructive pulmonary disease, chronic alcoholism | 99 |
| Antibiotic resistance rate (%) | 23 for ESBL-producing strains, 11 for carbapenemase-producing strains | | 13 |
| Primary strain type(s) | Classical | Classical and HV | 32, 41, 42, 48, 49, 60–62, 69 |

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 "currant jelly sputum," which is the production of thick blood-tinged mucous 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 for bacteria. Surfactants and immunoglobulins can also serve as opsonins for phagocytosis. In the lungs, surfactant protein A (SP-A) and a portion of the SP-B proprotein can act synergistically to enhance *K. pneumoniae* killing and to promote neutrophil recruitment (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).

Innate 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 of 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 pyronecrosis (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

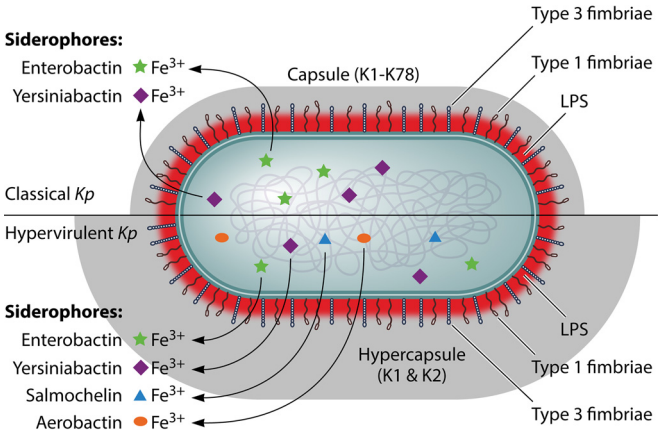


**FIG 1** Four well-characterized virulence factors in classical and hypervirulent *K. pneumoniae* (*Kp*) strains. There are four well-characterized virulence factors for pathogenic *K. pneumoniae*: capsule, LPS, fimbriae (type 1 and type 3), and siderophores. Capsule is an extracellular polysaccharide 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 pathogenicity. HV strains make a hypercapsule, which amplifies the production of capsular material, resulting in a relatively larger capsule, and are predominantly 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 fimbriae, and secrete iron-scavenging siderophores. Of the siderophores, enterobactin is made by almost all strains, and yersiniabactin is made by approximately half of classical and almost all HV strains. Salmochelin and aerobactin are rarely produced by classical strains but are typically secreted by HV strains, with aerobactin being the most highly expressed of the siderophores.

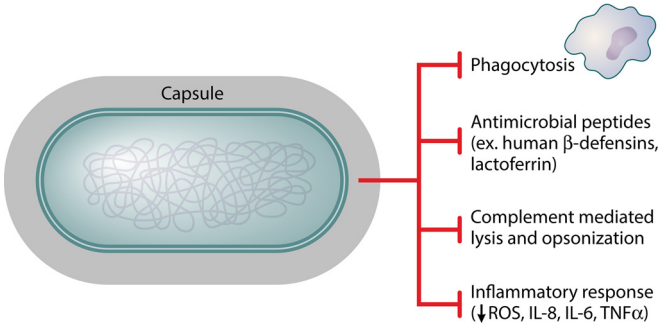


**FIG 2** Role of capsule in *K. pneumoniae* virulence. A number of different functions for capsule have been delineated for *K. pneumoniae* virulence. First, capsule prevents phagocytosis and opsonophagocytosis of the bacteria by immune cells. Second, it hinders the bactericidal action of antimicrobial 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 activation of the immune response, as measured by decreased reactive oxygen species (ROS), IL-8, IL-6, and TNF-α production, by assisting 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 compared 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 *galF* (204, 205). K antigens have been traditionally assigned by using serological methods (201). Recently, however, K-antigen typing is often performed by sequencing 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 encodes a surface protein involved in capsule attachment to the outer membrane, where the loss of this protein results in essentially 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 sequence 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 mucoid phenotype A (*rmpA*) and *rmpA2*; the expression of the chromosomal 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 result 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 chromosomal mucoviscosity-associated gene A (*magA*) (41). *magA* was discovered 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 $K_2$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 serotypes, 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 deterrent 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).

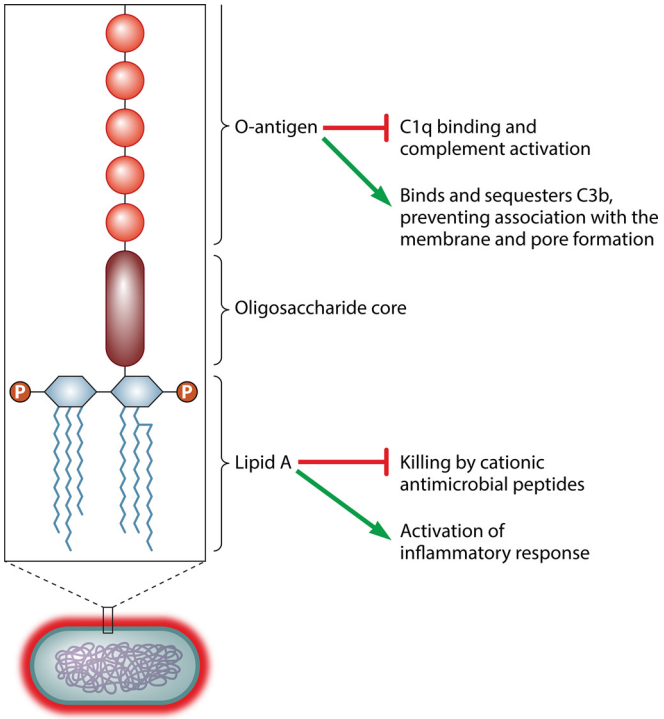


**FIG 3** Role of lipopolysaccharide in *K. pneumoniae* virulence. LPS is composed of three major subunits: lipid A, an oligosaccharide core, and O antigen. Lipid A inserts into the bacterial membrane and is a potentially potent activator of inflammation. *K. pneumoniae* may modify its lipid A to make it less inflammatory during infection, and lipid A may also protect against the bactericidal action of cationic antimicrobial peptides. O antigen is the outermost subunit of LPS. It has important roles in protecting against complement, including 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 component 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 *wb*, *waa*, and *lpx* gene clusters, respectively (253–256). Unlike the 77 different K antigens that have been documented for *K. pneumoniae* capsule, there have been only 9 different O-antigen types identified in *K. pneumoniae* isolates, and O1 is the most common (257).

LPS is both a benefit and a hindrance for *K. pneumoniae* during infection, as it is an important virulence factor that protects against humoral defenses but also can be a strong immune activator. 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, including neutrophils and macrophages, which clear *K. pneumoniae* infection and control spread to other tissues. This has been demonstrated in mouse models of *K. pneumoniae* bacterial infection, where mice lacking TLR4 or MyD88, a signaling protein downstream of TLR4, are more susceptible to *K. pneumoniae* pneumonia and systemic spread (170, 178, 258). In these more susceptible mice, there is impaired cytokine and chemokine production and recruitment of neutrophils. Certain *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 *K. pneumoniae* strains, where the hypercapsule dampens TLR4 signaling (259). Another method of preventing recognition by the immune response employed by some bacteria, such as *Yersinia pestis*, *Helicobacter pylori*, and *Porphyromonas gingivalis*, is modification of the LPS to a form that is no longer recognizable by certain immune receptors (260–262). Recent evidence suggests that *K. pneumoniae* may do the same (263). *K. pneumoniae* demonstrates 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 *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 *in vivo* virulence of *K. pneumoniae* (260, 263). Furthermore, the lipid A portion of *K. pneumoniae* LPS also plays a beneficial role in virulence, as a mutant strain of *K. pneumoniae* with altered lipid A acylation was attenuated in a mouse model of pneumonia. Additionally, both *in vitro* and *in vivo* experiments have shown that lipid A protects against some cationic antimicrobial peptides (152, 263).

*K. pneumoniae* is recognized by and activates the classical, alternative, and lectin complement pathways, although there is heterogeneity among which pathways become activated across *K. pneumoniae* 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-resistant strains activate the complement cascade but are not susceptible to killing due to the O-antigen portion of LPS. The O antigen protects against C3 by binding C3b, a complement component that is both an opsonin and part of the pore-forming process, 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, *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 *K. pneumoniae* sensitive to complement-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 *uge*, which encodes a UDP galacturonate 4-epimerase, and *wabG*, which encodes a GalA transferase (267, 268). The *uge* gene is present in the majority of *K. pneumoniae* isolates, including both disease-causing and commensal strains, and in the absence of this gene, *K. pneumoniae* LPS produces rough LPS and is less capable of causing UTI, pneumonia, and sepsis than WT strains (267, 269, 270). Meanwhile, *wabG* is likely present in almost all clinical isolates, with the ma-

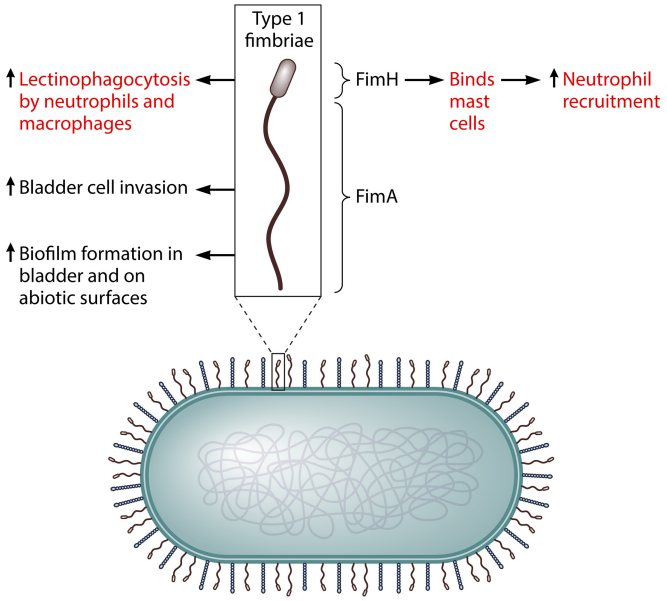


**FIG 4** Functions of type 1 fimbriae during *K. pneumoniae* infection and biofilm formation. Type 1 fimbriae are filamentous, membrane-bound, adhesive structures composed primarily of FimA subunits, with the FimH subunit on the tip. These fimbriae have a role in bladder cell invasion by *K. pneumoniae* as well as biofilm formation in the bladder and on abiotic surfaces. However, type 1 fimbriae may be a negative influence on *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.

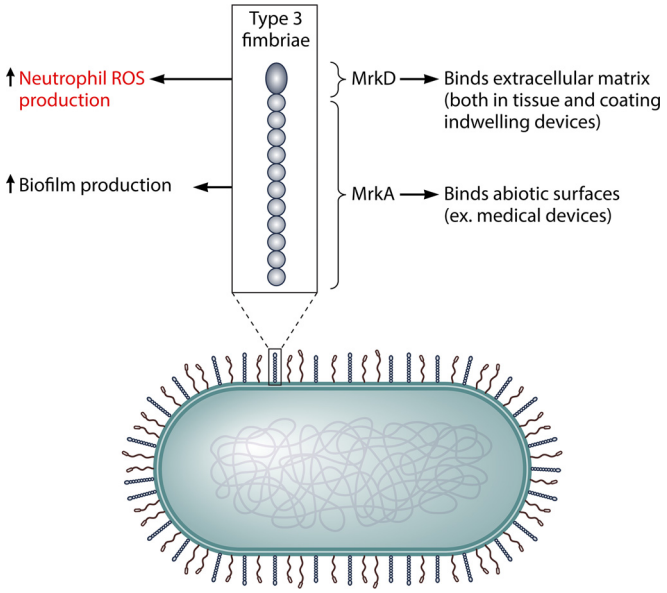


**FIG 5** Functions of type 3 fimbriae during *K. pneumoniae* infection and biofilm formation. Type 3 fimbriae are helix-like, membrane-bound, adhesive structures on the surface of *K. pneumoniae*. They are composed primarily of MrkA subunits, with the MrkD subunit on the tip. Type 3 fimbriae have been 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.

jority of reports stating that *wabG* is harbored by 88 to 100% of *K. pneumoniae* isolates, although one report found *wabG* in only 5.3% of isolates (268, 270–274). *K. pneumoniae* strains lacking this gene are unable to generate the LPS outer core or to retain capsular antigen and are attenuated in intraperitoneal, pneumonic, and UTI rodent models of infection (275).

### Type 1 and 3 Fimbriae

Fimbriae represent another class of *K. pneumoniae* virulence factors and are important mediators of *K. pneumoniae* adhesion (Fig. 4 and 5). In *K. pneumoniae*, type 1 and 3 fimbriae are the major adhesive structures that have been characterized as pathogenicity factors. Four other adhesive structures have been noted for *K. pneumoniae*, including another fimbria called KPF-28, a nonfimbrial factor called CF29K, and a capsule-like material (276–278). These structures were found to confer binding of *K. pneumoniae* to human carcinoma or intestinal cell lines, suggesting an involvement of these genes in GI tract colonization (276). One study found the expression of KPF-28 in 30/78 nosocomial isolates and CF29K in 3/85 *K. pneumoniae* nosocomial isolates (18, 276, 277). However, little has since been done to characterize the structures of CF29K or KPF-28 or their functions in animal model systems. Therefore, this section focuses on type 1 and 3 fimbriae and their role in virulence during infection.

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 and FimG; *fimC*, which encodes the fimbrial chaperone; *fimD*, which encodes an usher protein; and *fimI*, which encodes an uncharacterized product necessary for type 1 fimbriae. *K. pneumoniae* also carries a gene not carried by *E. coli*, *fimK*, whose exact function is unknown. One theory is that FimK is involved in type 1 fimbrial regulation, due to the loss of type 1 fimbria expression upon deletion of *fimK* (116, 283). *K. pneumoniae* type 1 fimbriae bind D-mannosylated glycoproteins, and therefore, binding by type 1 fimbriae is frequently termed "mannose-sensitive" binding (284, 285).

Type 3 fimbriae are helix-like filaments. In *K. pneumoniae*, these fimbriae are encoded by the *mrkABCD* gene cluster (286). The bulk of the structure consists of MrkA subunits, with the adhesin MrkD being located at the tip (Fig. 5). MrkB, -C, and -E are involved in assembly and the regulation of expression, while MrkF is involved in the surface stability of the fimbriae (287). In a manner similar to that of 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, 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). Typically, a biofilm forms when planktonically grown bacteria come into contact with a surface, adhere, and then form a complex three-dimensional (3D) structure. Bacteria within this structure often become resistant to chemicals to which planktonically grown bacteria may be susceptible (293). Biofilm structures can then give rise to planktonically growing bacteria that provide a source of infections. In *K. pneumoniae*, neither type of fimbria is expressed while *K. pneumoniae* is planktonic; however, type 3 fimbriae are expressed during biofilm formation on catheters, while the expression of type 1 fimbriae is controversial (294, 295). Therefore, one can speculate that these fimbriae may contribute to the occurrence of UTIs. In other *K. pneumoniae* biofilm models, type 3 fimbriae in particular have been shown to be crucial for biofilm production (289, 295, 296). The precise role of type 1 fimbriae in the production of biofilms by *K. pneumoniae* is not as clear. While one report found that type 1 fimbriae promoted *K. pneumoniae* biofilm formation on abiotic surfaces in a role reminiscent of that played by the homologous FimH protein in biofilm production by *E. coli*, another study found that type 1 fimbriae were downregulated in biofilm cells and did not influence biofilm formation (117, 295, 297, 298).

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

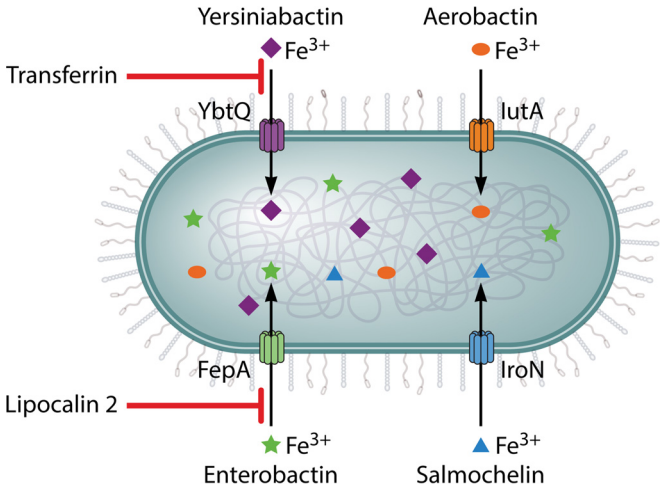


**FIG 6** Siderophore production and roles in virulence in *K. pneumoniae*. The ability to acquire iron in an iron-poor environment during infection is necessary for *K. pneumoniae* pathogenesis. Therefore, bacteria secrete proteins with a high affinity for iron, called siderophores. *K. pneumoniae* strains have been found to produce one or more of the following siderophores: enterobactin, salmochelin, yersiniabactin, and aerobactin. Enterobactin is the primary siderophore used by *K. pneumoniae*, although it is inhibited by the host molecule lipocalin-2. Salmochelin is a c-glucosylated form of enterobactin that can no longer be inhibited by lipocalin-2. Yersiniabactin and aerobactin are structurally distinct from enterobactin and salmochelin. Neither siderophore can be inhibited by lipocalin-2, but yersiniabactin functionality is reduced in the presence of the host molecule transferrin. The production of a number of different siderophores may allow *K. pneumoniae* to colonize and disseminate to a number of different sites within the host, with niche-specific roles for each siderophore. FepA, IroN, YbtQ, and IutA serve as transporters specific to their corresponding siderophores of enterobactin, salmochelin, yersiniabactin, and aerobactin, respectively.

in phagocytosis are not as clear as with type 1 fimbriae, *in vitro* work has shown that *K. pneumoniae* type 3 fimbriae can stimulate an oxidative response in neutrophils (306).

## 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 of 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 classical 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, 321, 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. pneu-*

*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, *iroBCDE* (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 abscess (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, *iucABCD*, along with the aerobactin transporter *iutA* 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 pneumonic 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 (353). However, porin downregulation may come at a fitness cost in terms of virulence (345). In an *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 can colonize the liver, but cannot persist, following intraperitoneal injection (345). Likewise, 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 upregulated 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 ($LD_{50}$) 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

**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[a]

| Strain(s) | Library size (no. of clones), type or method | Infection model(s) | Tissue(s) studied | No. of hits | Gene(s) of note | Reference |
|---|---|---|---|---|---|---|
| *K. pneumoniae* ATCC 43816 Rif$^r$ (KPPR1) | 4,800, STM | *In vivo* screen, intranasal, C57BL/6 mice | Lungs, spleen | 106 | *wecA*, *wzyE*, *rfbB* | 167 |
| *K. pneumoniae* ATCC 43816 Rif$^r$ (KPPR1) | 25,000, Tn mutagenesis | *In vivo* screen, retropharyngeal, C57BL/6 mice | Lungs | >300 | *rfaH*, *aroE*, *ilvCD*, *copA* | 363 |
| *K. pneumoniae* ATCC 43816, *K. pneumoniae* IA565 | NA, subtractive hybridization | *In vivo* screen, intratracheal, C57BL/6 mice | Lungs, blood | 9 | *iroN*, *dcoB*, *orf350*, *kvgS*, *ybiT*, *mrr*, *fimD*, *sthB* | 168 |
| *K. pneumoniae* 52145, *K. pneumoniae* SB2390, *K. pneumoniae* SB3193 | NA, comparison of *K. pneumoniae* genomes | *In silico* analysis, genomic sequence analysis; *in vivo* follow-up, intranasal, BALB/c mice | NA | NA | *pld1*, *waa* | 367 |
| *K. pneumoniae* 52145 | 5,320, Tn mutagenesis | *In vitro* screen, NF-κB reporter A549 cell line; *in vivo* follow-up, intranasal, CD-1 mice | NA | 114 | *waa*, *pulA* | 368 |
| *K. pneumoniae* ATCC 43816 | 2,000, Tn mutagenesis | *In vitro* screen, biofilm formation; *in vivo* follow-up, intranasal and intraperitoneal, mice | NA | 4 | *yciI*, *yadH*, *yhdH*, *pduA* | 369 |
| *K. pneumoniae* ATCC 43816 | 1,175, STM | *In vitro* screen, biofilm formation; *in vivo* follow-up, intranasal, BALB/c mice | NA | 8 | *luxR*, *lysR*, *crp*, *orf12*, *fimA* | 370 |
| *K. pneumoniae* LM21 | 2,200, STM | *In vivo* screen, oral, IOPS mice; *in vitro* follow-up, intestinal cell line | Colon | 29 | *ntrC*, *fmdD*, *glgP*, *fucA-fucP*, *fruB-pftA* | 364 |
| *K. pneumoniae* 3091, *K. pneumoniae* C3091 Sm$^r$ Rif$^r$ | 1,440, STM | *In vivo* screen, oral and transurethral, Ssc,CF1 mice | Feces, bladder | 19 | *waaL*, *waaE*, *wbbO*, *plsX*, *ompA*, *surA*, *tufA*, *hupA*, *arcB*, *gmd*, *fcl*, *fimB* | 365 |
| NTUH-K2044 (HV) | 2,500, Tn mutagenesis | *In vitro* screen, biofilm formation; *in vivo* follow-up, intragastric, BALB/c mice | Colon | 27 | *treC*, *sugE*, *wza* | 371 |
| *K. pneumoniae* CG43 (HV) | 2,880, STM | *In vivo* screen, oral, BALB/c mice | Liver, spleen | 28 | *ymdF* | 366 |

[a] Abbreviations: NA, not applicable; STM, signature-tagged mutagenesis; Tn, transposon; Rif$^r$, rifampin resistant; Sm$^r$, streptomycin resistant.

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 (363). 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 (363).

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*, *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* K2 genome of Kp52.145 (the strain in which *rmpA* and the aerobactin cluster were originally identified as virulence factors in *K. pneumoniae*) with the sequences of the low-virulence K1 SB3193 and K2 SB2390 strains as well as with the sequences of the well-characterized virulent reference K1 NTUH-2044 and K5 MGH78578 strains (215, 336, 372). From this work, five genomic regions with pathogenic features were identified in the newly sequenced HV K2 strain. One region encoded yersiniabactin, colibactin, and a type IV secretion system; a second encoded a putative cytotoxic outer membrane

**TABLE 4** *K. pneumoniae* genes involved in virulence identified and/or confirmed in *in vivo* mouse models[a]

| Category and gene | Infection model(s) | Strain | Putative function | Defect(s) in *in vivo* screen | Defect in 1:1 comparison | Defect in single strain | Reference |
|---|---|---|---|---|---|---|---|
| LPS | | | | | | | |
| *waaL* | GI and UTI | C3091 | LPS core synthesis | GI and UTI | UTI | NA | 365 |
| *waaE* | GI and UTI | C3091 | LPS core synthesis | GI and UTI | UTI | NA | 365 |
| *wbbO* | GI and UTI | C3091 | O-antigen synthesis | GI and UTI | UTI | NA | 365 |
| *waaL* | Pneumonia | 52145 | LPS core synthesis | NA | NA | Pneumonia | 368 |
| *rfbB* | Pneumonia | KPPR1 | O-antigen export | Pneumonia | NA | Pneumonia | 167 |
| *wecA* | Pneumonia | KPPR1 | GlcNAc-1-phosphate transferase | Pneumonia | NA | Pneumonia | 167 |
| Capsule | | | | | | | |
| *gmd* | UTI | C3091 | Fucose synthesis | UTI | UTI | NA | 365 |
| *fcl* | UTI | C3091 | Fucose synthesis | UTI | UTI | NA | 365 |
| ORF12 | Pneumonia | ATCC 43816 | Capsule synthesis | NA | NA | Pneumonia | 370 |
| *cpsB* | Pneumonia | KPPR1 | Mannose-1-phosphate guanyltransferase | Pneumonia | NA | Pneumonia | 167 |
| *wzi* | Pneumonia | KPPR1 | Capsule assembly Wzi family protein | Pneumonia | NA | NA | 363 |
| *wcaJ* | Pneumonia | KPPR1 | Undecaprenyl-phosphate glucose phosphotransferase | Pneumonia | NA | NA | 363 |
| *rcsB* | Pneumonia | KPPR1 | Transcriptional regulator | Pneumonia | NA | NA | 363 |
| Adhesion | | | | | | | |
| *hmw1A* | GI | LM21 | Surface protein | GI | GI | NA | 364 |
| *fimB* | UTI | C3091 | Type I fimbriae regulation | UTI | UTI | NA | 365 |
| *mrkC* | Liver abscess | CG43 | Usher protein of type III fimbriae | Liver abscess | NA | Liver abscess* | 366 |
| *fimC* | Liver abscess | CG43 | Chaperone protein of type I fimbriae | Liver abscess | NA | Liver abscess* | 366 |
| *fimA* homologue | Pneumonia | ATCC 43816 | Fimbrial synthesis | NA | NA | Pneumonia | 370 |
| Metabolism | | | | | | | |
| *arcB* | GI and UTI | C3091 | Regulation of aerobe-anaerobe metabolism | GI and UTI | UTI | NA | 365 |
| *ygjD* | GI | LM21 | *O*-Sialoglycoprotein endopeptidase | GI | GI | NA | 364 |
| *lacI-lacZ* | GI | LM21 | Lactose metabolic enzyme | GI | GI | NA | 364 |
| *pheC* | GI | LM21 | Cyclohexadienyl dehydratase | GI | GI | NA | 364 |
| *glgP* | GI | LM21 | Alpha-glucan phosphorylase | GI | GI | NA | 364 |
| *PA2698* | GI | LM21 | Hydrolase | GI | GI | NA | 364 |
| *treC* | GI | NTUH-K2044 | Trehalose-6-phosphate hydrolase | NA | GI | NA | 371 |
| *galK* | Liver abscess | CG43 | Galactokinase | Liver abscess | NA | Liver abscess* | 366 |
| *proV* | Liver abscess | CG43 | Glycine betaine/L-proline ABC transporter | Liver abscess | NA | Liver abscess* | 366 |
| *araF* | Liver abscess | CG43 | L-Arabinose-binding periplasmic protein | Liver abscess | NA | Liver abscess* | 366 |
| *rhaB* | Liver abscess | CG43 | Putative α-L-rhamnosidase | Liver abscess | NA | Liver abscess* | 366 |
| *gabD* | Liver abscess | CG43 | Succinate-semialdehyde dehydrogenase | Liver abscess | NA | Liver abscess* | 366 |
| *pgdH* | Liver abscess | CG43 | D-3 phosphoglycerate dehydrogenase | Liver abscess | NA | Liver abscess* | 366 |
| *galT* | Liver abscess | CG43 | Galactose-1-phosphate uridylyltransferase | Liver abscess | NA | Liver abscess* | 366 |
| *ahpC* | Liver abscess | CG43 | Alkyl hydroperoxide reductase | Liver abscess | NA | Liver abscess* | 366 |
| *lyxK* | Liver abscess | CG43 | Putative L-xylulokinase | Liver abscess | NA | Liver abscess* | 366 |
| *ilvC* | Pneumonia | KPPR1 | Ketol acid reductoisomerase | Pneumonia | NA | Pneumonia | 363 |
| *ilvD* | Pneumonia | KPPR1 | Dihydroxy acid dehydratase | Pneumonia | NA | Pneumonia | 363 |
| *aroE* | Pneumonia | KPPR1 | Dehydroshikimate reductase | Pneumonia | NA | Pneumonia | 363 |
| *argR* | Pneumonia | KPPR1 | Arginine repressor | Pneumonia | NA | NA | 363 |
| *ilvE* | Pneumonia | KPPR1 | Branched-chain-amino-acid aminotransferase | Pneumonia | NA | NA | 363 |

(Continued on following page)

**TABLE 4** (Continued)

| Category and gene | Infection model(s) | Strain | Putative function | Defect(s) in *in vivo* screen | Defect in 1:1 comparison | Defect in single strain | Reference |
|---|---|---|---|---|---|---|---|
| *purF* | Pneumonia | KPPR1 | Amidophosphoribosyl-transferase | Pneumonia | NA | NA | 363 |
| *purI* | Pneumonia | KPPR1 | Phosphoribosylformylgly-cinamidine synthase | Pneumonia | NA | NA | 363 |
| *trpD* | Pneumonia | KPPR1 | Anthranilate synthase component II | Pneumonia | NA | NA | 363 |
| *serA* | Pneumonia | KPPR1 | D-3 phosphoglycerate dehydrogenase | Pneumonia | NA | NA | 363 |
| *leuC* | Pneumonia | KPPR1 | 3-Isopropylmalate isomerase subunit | Pneumonia | NA | NA | 363 |
| *pheA* | Pneumonia | KPPR1 | Bifunctional chorismate mutase/prephenate dehydratase | Pneumonia | NA | NA | 363 |
| *purH* | Pneumonia | KPPR1 | Phosphoribosylaminoimi-dazole carboxamide formyltransferase | Pneumonia | NA | NA | 363 |
| *dgo* | Pneumonia | KPPR1 | 2-Oxo-3-deoxygalactonate 6-phosphate aldolase | Pneumonia | NA | NA | 363 |
| *pld1* | Pneumonia | 52.145 | Putative cardiolipin synthase | NA | NA | Pneumonia | 367 |
| *pulA* | Pneumonia | 52145 | Pullulan-degrading glucanase | NA | NA | Pneumonia | 368 |
| Cell surface | | | | | | | |
| *ompA* | GI and UTI | C3091 | Outer membrane protein | GI and UTI | UTI | NA | 365 |
| *surA* | GI and UTI | C3091 | Folding of outer membrane proteins | GI and UTI | UTI | NA | 365 |
| *hgpA* | Liver abscess | CG43 | Hemoglobulin-binding protein | Liver abscess | NA | Liver abscess* | 366 |
| *pls* | Liver abscess | CG43 | Surface large repetitive protein | Liver abscess | NA | Liver abscess* | 366 |
| *pteA* | Liver abscess | CG43 | Cellobiose-specific phosphotransferase IIA | Liver abscess | NA | Liver abscess* | 366 |
| *pteC* | Liver abscess | CG43 | Cellobiose-specific phosphotransferase IIC | Liver abscess | NA | Liver abscess* | 366 |
| *pagO* | Liver abscess | CG43 | PhoPQ-activated integral membrane protein | Liver abscess | NA | Liver abscess* | 366 |
| *uraA* | Liver abscess | CG43 | Uracil permease | Liver abscess | NA | Liver abscess* | 366 |
| Lipid synthesis | | | | | | | |
| *plsX* | GI and UTI | C3091 | Fatty acid and phospholipid synthesis | GI and UTI | UTI | NA | 365 |
| Protein synthesis | | | | | | | |
| *tufA* | GI and UTI | C3091 | Protein synthesis elongation factor | GI and UTI | UTI | NA | 365 |
| Nucleic acid related | | | | | | | |
| *hupA* | GI and UTI | C3091 | DNA folding | GI and UTI | UTI | NA | 365 |
| ECso303 | GI | LM21 | DNA primase | GI | GI | NA | 364 |
| *prmB* | GI | LM21 | Adenine-specific methylase | GI | GI | NA | 364 |
| *int* | Liver abscess | CG43 | Putative integrase | Liver abscess | | Liver abscess* | 366 |
| Transporters | | | | | | | |
| *fimD* | GI | LM21 | Amide-urea-binding protein | GI | GI | NA | 364 |
| *hrcU* | GI | LM21 | Hairpin type III secretion system | GI | GI | NA | 364 |
| *copA* | Pneumonia | KPPR1 | Copper-translocating P-type ATPase | Pneumonia | NA | Pneumonia | 363 |
| *tatC* | Pneumonia | KPPR1 | Twin-arginine-targeting protein translocase | Pneumonia | NA | NA | 363 |
| Regulators | | | | | | | |
| *ntrC* | GI | LM21 | Nitrogen metabolism regulator | GI | GI | NA | 364 |
| *gcvR* | GI | LM21 | Glycine metabolism regulator | GI | GI | NA | 364 |

(Continued on following page)

**TABLE 4** (Continued)

| Category and gene | Infection model(s) | Strain | Putative function | Defect(s) in *in vivo* screen | Defect in 1:1 comparison | Defect in single strain | Reference |
|---|---|---|---|---|---|---|---|
| *cbl* | Liver abscess | CG43 | HTH-type transcriptional regulator | Liver abscess | NA | Liver abscess* | 366 |
| *evgA* | Liver abscess | CG43 | Response regulator of two-component system | Liver abscess | NA | Liver abscess* | 366 |
| *moaR* | Liver abscess | CG43 | Monoamine regulon positive regulator | Liver abscess | NA | Liver abscess* | 366 |
| *evgS* | Liver abscess | CG43 | Histidine kinase sensor | Liver abscess | NA | Liver abscess* | 366 |
| *esgD* | Liver abscess | CG43 | Response regulator for second curli | Liver abscess | NA | Liver abscess* | 366 |
| *kva15* | Liver abscess | CG43 | Putative LuxR family transcriptional regulator | Liver abscess | NA | Liver abscess* | 366 |
| *kva19* | Liver abscess | CG43 | Putative UphA family transcriptional regulator | Liver abscess | NA | Liver abscess* | 366 |
| *rfaH* | Pneumonia | KPPR1 | Transcriptional activator | Pneumonia | NA | Pneumonia | 363 |
| *galF* | Pneumonia | KPPR1 | Regulatory protein | Pneumonia | NA | NA | 363 |
| *phoR* | Pneumonia | KPPR1 | Phosphate regulon sensor kinase | Pneumonia | NA | NA | 363 |
| *VK055_4417* | Pneumonia | KPPR1 | MarR family transcriptional regulator | Pneumonia | NA | Pneumonia | 363 |
| *luxR* homologue | Pneumonia | ATCC 43816 | Transcriptional regulation | NA | NA | Pneumonia | 370 |
| *lysR* homologue | Pneumonia | ATCC 43816 | Transcriptional regulation | NA | NA | Pneumonia | 370 |
| CRP homologue | Pneumonia | ATCC 43816 | Transcriptional regulation | NA | NA | Pneumonia | 370 |
| Hypothetical | | | | | | | |
| ORF *b2512* | GI and UTI | C3091 | Hypothetical protein | GI and UTI | UTI | NA | 365 |
| ORF *b1631* | GI and UTI | C3091 | Hypothetical protein | GI and UTI | UTI | NA | 365 |
| ORF *ytfN* | UTI | C3091 | Hypothetical protein | UTI | UTI | NA | 365 |
| *VK055_5096* | Pneumonia | KPPR1 | Hypothetical protein | Pneumonia | NA | NA | 363 |
| *VK055_5023* | Pneumonia | KPPR1 | Hypothetical protein | Pneumonia | NA | NA | 363 |
| *VK055_3515* | Pneumonia | KPPR1 | Hypothetical protein | Pneumonia | NA | NA | 363 |
| *kva7* | Liver abscess | CG43 | Hypothetical protein | Liver abscess | NA | Liver abscess* | 366 |
| *yfgG* | Liver abscess | CG43 | Hypothetical protein | Liver abscess | NA | Liver abscess* | 366 |
| *ymdF* | Liver abscess | CG43 | Hypothetical protein | Liver abscess | NA | Liver abscess* | 366 |
| Unknown | | | | | | | |
| *yebE* | GI | LM21 | DUF533 family inner membrane protein | GI | GI | NA | 364 |
| *yciI* | Pneumonia | ATCC 43816 | Putative DGPF domain-containing enzyme | NA | NA | Pneumonia | 369 |

[a] 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. (167), 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.

protein; a third encoded proteins putatively involved in adhesion and evasion of the immune response; a fourth encoded a number of phage-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

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*, *yhdH*, 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*, *lysR*, and *crp*), one gene is important for capsule synthesis (*orf12*), and one gene is important for fimbria 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 EIIB and EIIC), and one mutant lacks a gene predicted to encode a protein of unknown function (YPTB1848). 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 (*plsX*, *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 L-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 fimbria 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 (*cspC*), 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.

## ACKNOWLEDGMENTS

We thank Erin R. Green for critical reading of this review.

This work was supported by NIH grant RO1AI113166 awarded to J.M. M.K.P. is a Howard Hughes Medical Institute (HHMI) Med into Grad Scholar supported in part by a grant to the Sackler School of Biomedical Sciences, Tufts University School of Medicine, from the HHMI through the Med into Grad Initiative. M.K.P. was also supported by grants 2T32AI007077-31 and T32AI007422. J.M. has a subcontract with Microbiotix on work involving *Pseudomonas*.

## REFERENCES

1. **Friedlander C.** 1882. Uber die scizomyceten bei der acuten fibrosen pneumonie. Arch Pathol Anat Physiol Klin Med **87:**319–324.
2. **Merino S, Camprubi S, Alberti S, Benedi VJ, Tomas JM.** 1992. Mechanisms of Klebsiella pneumoniae resistance to complement-mediated killing. Infect Immun **60:**2529–2535.
3. **Bagley ST.** 1985. Habitat association of Klebsiella species. Infect Control **6:**52–58.
4. **Rock C, Thom KA, Masnick M, Johnson JK, Harris AD, Morgan DJ.** 2014. Frequency of Klebsiella pneumoniae carbapenemase (KPC)-producing and non-KPC-producing Klebsiella species contamination of healthcare workers and the environment. Infect Control Hosp Epidemiol **35:**426–429. http://dx.doi.org/10.1086/675598.
5. **Dao TT, Liebenthal D, Tran TK, Ngoc Thi Vu B, Ngoc Thi Nguyen D, Thi Tran HK, Thi Nguyen CK, Thi Vu HL, Fox A, Horby P, Van Nguyen K, Wertheim HFL.** 2014. Klebsiella pneumoniae oropharyngeal carriage in rural and urban Vietnam and the effect of alcohol consumption. PLoS One **9:**e91999. http://dx.doi.org/10.1371/journal.pone.0091999.
6. **Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J.** 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis **48:**1–12. http://dx.doi.org/10.1086/595011.
7. **Kuehn BM.** 2013. "Nightmare" bacteria on the rise in US hospitals, long-term care facilities. JAMA **309:**1573–1574. http://dx.doi.org/10.1001/jama.2013.2922.
8. **CDC.** 2015. CDC works 24/7 to protect US from health, safety and security threats. CDC, Atlanta, GA.
9. **Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC.** 2001. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother **45:**1151–1161. http://dx.doi.org/10.1128/AAC.45.4.1151-1161.2001.
10. **Pitout JD, Nordmann P, Poirel L.** 2015. Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother **59:**5873–5884. http://dx.doi.org/10.1128/AAC.01019-15.
11. **Iredell J, Brown J, Tagg K.** 2016. Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. BMJ **352:**h6420. http://dx.doi.org/10.1136/bmj.h6420.

12. **Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP.** 2013. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis **13:**785–796. http://dx.doi.org/10.1016/S1473-3099(13)70190-7.
13. **CDC.** 2013. Vital signs: carbapenem-resistant Enterobacteriaceae. MMWR Morb Mortal Wkly Rep **62:**165–170.
14. **Chen L, Mathema B, Chavda KD, DeLeo FR, Bonomo RA, Kreiswirth BN.** 2014. Carbapenemase-producing Klebsiella pneumoniae: molecular and genetic decoding. Trends Microbiol **22:**686–696. http://dx.doi.org/10.1016/j.tim.2014.09.003.
15. **Chen L, Mathema B, Pitout JD, DeLeo FR, Kreiswirth BN.** 2014. Epidemic Klebsiella pneumoniae ST258 is a hybrid strain. mBio **5:**e01355-14. http://dx.doi.org/10.1128/mBio.01355-14.
16. **Cuzon G, Naas T, Truong H, Villegas MV, Wisell KT, Carmeli Y, Gales AC, Venezia SN, Quinn JP, Nordmann P.** 2010. Worldwide diversity of Klebsiella pneumoniae that produce beta-lactamase blaKPC-2 gene. Emerg Infect Dis **16:**1349–1356. http://dx.doi.org/10.3201/eid1609.091389.
17. **Deleo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, Chavda KD, Jacobs MR, Mathema B, Olsen RJ, Bonomo RA, Musser JM, Kreiswirth BN.** 2014. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 Klebsiella pneumoniae. Proc Natl Acad Sci U S A **111:**4988–4993. http://dx.doi.org/10.1073/pnas.1321364111.
18. **Brisse S, Fevre C, Passet V, Issenhuth-Jeanjean S, Tournebize R, Diancourt L, Grimont P.** 2009. Virulent clones of Klebsiella pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS One **4:**e4982. http://dx.doi.org/10.1371/journal.pone.0004982.
19. **Adler A, Khabra E, Chmelnitsky I, Giakkoupi P, Vatopoulos A, Mathers AJ, Yeh AJ, Sifri CD, De Angelis G, Tacconelli E, Villegas MV, Quinn J, Carmeli Y.** 2014. Development and validation of a multiplex PCR assay for identification of the epidemic ST-258/512 KPC-producing Klebsiella pneumoniae clone. Diagn Microbiol Infect Dis **78:**12–15. http://dx.doi.org/10.1016/j.diagmicrobio.2013.10.003.
20. **Chmelnitsky I, Shklyar M, Hermesh O, Navon-Venezia S, Edgar R, Carmeli Y.** 2013. Unique genes identified in the epidemic extremely drug-resistant KPC-producing Klebsiella pneumoniae sequence type 258. J Antimicrob Chemother **68:**74–83. http://dx.doi.org/10.1093/jac/dks370.
21. **Tzouvelekis LS, Miriagou V, Kotsakis SD, Spyridopoulou K, Athanasiou E, Karagouni E, Tzelepi E, Daikos GL.** 2013. KPC-producing, multidrug-resistant Klebsiella pneumoniae sequence type 258 as a typical opportunistic pathogen. Antimicrob Agents Chemother **57:**5144–5146. http://dx.doi.org/10.1128/AAC.01052-13.
22. **Xiong H, Carter RA, Leiner IM, Tang YW, Chen L, Kreiswirth BN, Pamer EG.** 8 June 2015. Distinct contributions of neutrophils and $CCR2^+$ monocytes to pulmonary clearance of different Klebsiella pneumoniae strains. Infect Immun http://dx.doi.org/10.1128/IAI.00678-15.
23. **Chiang TT, Yang YS, Yeh KM, Chiu SK, Wang NC, Lin TY, Huang LY, Chang FY, Siu LK, Lin JC, Chen JH.** 2016. Quantification and comparison of virulence and characteristics of different variants of carbapenemase-producing Klebsiella pneumoniae clinical isolates from Taiwan and the United States. J Microbiol Immunol Infect **49:**83–90. http://dx.doi.org/10.1016/j.jmii.2015.08.011.
24. **Kobayashi SD, Porter AR, Dorward DW, Brinkworth AJ, Chen L, Kreiswirth BN, DeLeo FR.** 14 January 2016. Phagocytosis and killing of carbapenem-resistant ST258 Klebsiella pneumoniae by human neutrophils. J Infect Dis http://dx.doi.org/10.1093/infdis/jiw001.
25. **Boyle DP, Zembower TR.** 2015. Epidemiology and management of emerging drug-resistant Gram-negative bacteria: extended-spectrum beta-lactamases and beyond. Urol Clin North Am **42:**493–505. http://dx.doi.org/10.1016/j.ucl.2015.05.005.
26. **Wang D, Chen J, Yang L, Mou Y, Yang Y.** 2014. Phenotypic and enzymatic comparative analysis of the KPC variants, KPC-2 and its recently discovered variant KPC-15. PLoS One **9:**e111491. http://dx.doi.org/10.1371/journal.pone.0111491.
27. **Nordmann P, Cuzon G, Naas T.** 2009. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis **9:**228–236. http://dx.doi.org/10.1016/S1473-3099(09)70054-4.
28. **Villegas MV, Lolans K, Correa A, Kattan JN, Lopez JA, Quinn JP.** 2007. First identification of Pseudomonas aeruginosa isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. Antimicrob Agents Chemother **51:**1553–1555. http://dx.doi.org/10.1128/AAC.01405-06.
29. **Cuzon G, Naas T, Correa A, Quinn JP, Villegas MV, Nordmann P.** 2013. Dissemination of the KPC-2 carbapenemase in non-Klebsiella pneumoniae enterobacterial isolates from Colombia. Int J Antimicrob Agents **42:**59–62. http://dx.doi.org/10.1016/j.ijantimicag.2013.04.002.
30. **Cuzon G, Naas T, Villegas MV, Correa A, Quinn JP, Nordmann P.** 2011. Wide dissemination of Pseudomonas aeruginosa producing beta-lactamase blaKPC-2 gene in Colombia. Antimicrob Agents Chemother **55:**5350–5353. http://dx.doi.org/10.1128/AAC.00297-11.
31. **Cuzon G, Naas T, Guibert M, Nordmann P.** 2010. In vivo selection of imipenem-resistant Klebsiella pneumoniae producing extended-spectrum beta-lactamase CTX-M-15 and plasmid-encoded DHA-1 cephalosporinase. Int J Antimicrob Agents **35:**265–268. http://dx.doi.org/10.1016/j.ijantimicag.2009.10.021.
32. **Tsay RW, Siu LK, Fung CP, Chang FY.** 2002. Characteristics of bacteremia between community-acquired and nosocomial Klebsiella pneumoniae infection: risk factor for mortality and the impact of capsular serotypes as a herald for community-acquired infection. Arch Intern Med **162:**1021–1027. http://dx.doi.org/10.1001/archinte.162.9.1021.
33. **Meatherall BL, Gregson D, Ross T, Pitout JD, Laupland KB.** 2009. Incidence, risk factors, and outcomes of Klebsiella pneumoniae bacteremia. Am J Med **122:**866–873. http://dx.doi.org/10.1016/j.amjmed.2009.03.034.
34. **Korvick JA, Hackett AK, Yu VL, Muder RR.** 1991. Klebsiella pneumonia in the modern era: clinicoradiographic correlations. South Med J **84:**200–204. http://dx.doi.org/10.1097/00007611-199102000-00012.
35. **Cheng DL, Liu YC, Yen MY, Liu CY, Wang RS.** 1991. Septic metastatic lesions of pyogenic liver abscess. Their association with Klebsiella pneumoniae bacteremia in diabetic patients. Arch Intern Med **151:**1557–1559.
36. **Liu YC, Cheng DL, Lin CL.** 1986. Klebsiella pneumoniae liver abscess associated with septic endophthalmitis. Arch Intern Med **146:**1913–1916. http://dx.doi.org/10.1001/archinte.1986.00360220057011.
37. **Patel PK, Russo TA, Karchmer AW.** 2014. Hypervirulent Klebsiella pneumoniae. Open Forum Infect Dis **1:**ofu028. http://dx.doi.org/10.1093/ofid/ofu028.
38. **Shon AS, Bajwa RP, Russo TA.** 2013. Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: a new and dangerous breed. Virulence **4:**107–118. http://dx.doi.org/10.4161/viru.22718.
39. **Nadasy KA, Domiati-Saad R, Tribble MA.** 2007. Invasive Klebsiella pneumoniae syndrome in North America. Clin Infect Dis **45:**e25–e28. http://dx.doi.org/10.1086/519424.
40. **Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Bonomo RA.** 2003. Extended-spectrum beta-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type beta-lactamases. Antimicrob Agents Chemother **47:**3554–3560. http://dx.doi.org/10.1128/AAC.47.11.3554-3560.2003.
41. **Yu WL, Ko WC, Cheng KC, Lee HC, Ke DS, Lee CC, Fung CP, Chuang YC.** 2006. Association between rmpA and magA genes and clinical syndromes caused by Klebsiella pneumoniae in Taiwan. Clin Infect Dis **42:**1351–1358. http://dx.doi.org/10.1086/503420.
42. **Ko WC, Paterson DL, Sagnimeni AJ, Hansen DS, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP, McCormack JG, Yu VL.** 2002. Community-acquired Klebsiella pneumoniae bacteremia: global differences in clinical patterns. Emerg Infect Dis **8:**160–166. http://dx.doi.org/10.3201/eid0802.010025.
43. **Yang PC, Luh KT, Lee YC, Chang DB, Yu CJ, Wu HD, Lee LN, Kuo SH.** 1991. Lung abscesses: US examination and US-guided transthoracic aspiration. Radiology **180:**171–175. http://dx.doi.org/10.1148/radiology.180.1.2052687.
44. **Wang JL, Chen KY, Fang CT, Hsueh PR, Yang PC, Chang SC.** 2005. Changing bacteriology of adult community-acquired lung abscess in Taiwan: Klebsiella pneumoniae versus anaerobes. Clin Infect Dis **40:**915–922. http://dx.doi.org/10.1086/428574.
45. **Zhang Y, Zeng J, Liu W, Zhao F, Hu Z, Zhao C, Wang Q, Wang X, Chen H, Li H, Zhang F, Li S, Cao B, Wang H.** 21 August 2015. Emergence of a hypervirulent carbapenem-resistant Klebsiella pneu-

moniae isolate from clinical infections in China. J Infect http://dx.doi.org/10.1016/j.jinf.2015.07.010.
46. **Su SC, Siu LK, Ma L, Yeh KM, Fung CP, Lin JC, Chang FY.** 2008. Community-acquired liver abscess caused by serotype K1 Klebsiella pneumoniae with CTX-M-15-type extended-spectrum beta-lactamase. Antimicrob Agents Chemother **52:**804–805. http://dx.doi.org/10.1128/AAC.01269-07.
47. **Li W, Sun G, Yu Y, Li N, Chen M, Jin R, Jiao Y, Wu H.** 2014. Increasing occurrence of antimicrobial-resistant hypervirulent (hypermucoviscous) Klebsiella pneumoniae isolates in China. Clin Infect Dis **58:**225–232. http://dx.doi.org/10.1093/cid/cit675.
48. **Russo TA, Shon AS, Beanan JM, Olson R, MacDonald U, Pomakov AO, Visitacion MP.** 2011. Hypervirulent K. pneumoniae secretes more and more active iron-acquisition molecules than "classical" K. pneumoniae thereby enhancing its virulence. PLoS One **6:**e26734. http://dx.doi.org/10.1371/journal.pone.0026734.
49. **Russo TA, Olson R, Macdonald U, Metzger D, Maltese LM, Drake EJ, Gulick AM.** 2014. Aerobactin mediates virulence and accounts for increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) Klebsiella pneumoniae. Infect Immun **82:**2356–2367. http://dx.doi.org/10.1128/IAI.01667-13.
50. **Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Hollod L, Nadle J, Ray SM, Thompson DL, Wilson LE, Fridkin SK.** 2014. Multistate point-prevalence survey of health care-associated infections. N Engl J Med **370:**1198–1208. http://dx.doi.org/10.1056/NEJMoa1306801.
51. **Kang C-I, Kim S-H, Bang J-W, Kim H-B, Kim N-J, Kim E-C, Oh M-D, Choe K-W.** 2006. Community-acquired versus nosocomial Klebsiella pneumoniae bacteremia: clinical features, treatment outcomes, and clinical implication of antimicrobial resistance. J Korean Med Sci **21:**816–822. http://dx.doi.org/10.3346/jkms.2006.21.5.816.
52. **Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, Polsky B, Adams-Haduch JM, Doi Y.** 2012. Treatment outcome of bacteremia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother **56:**2108–2113. http://dx.doi.org/10.1128/AAC.06268-11.
53. **Zarkotou O, Pournaras S, Tselioti P, Dragoumanos V, Pitiriga V, Ranellou K, Prekates A, Themeli-Digalaki K, Tsakris A.** 2011. Predictors of mortality in patients with bloodstream infections caused by KPC-producing Klebsiella pneumoniae and impact of appropriate antimicrobial treatment. Clin Microbiol Infect **17:**1798–1803. http://dx.doi.org/10.1111/j.1469-0691.2011.03514.x.
54. **Bassi GL, Ferrer M, Torres A.** 2011. Nosocomial pneumonia, p 464–480. *In* Vincent J-L, Abraham E, Moore FA, Kochanek PM, Fink MP (ed), Textbook of critical care, 6th ed. Elsevier Saunders, Philadelphia, PA.
55. **Rotstein C, Evans G, Born A, Grossman R, Light RB, Magder S, McTaggart B, Weiss K, Zhanel GG.** 2008. Clinical practice guidelines for hospital-acquired pneumonia and ventilator-associated pneumonia in adults. Can J Infect Dis Med Microbiol **19:**19–53.
56. **Pingleton SK, Fagon JY, Leeper KV, Jr.** 1992. Patient selection for clinical investigation of ventilator-associated pneumonia. Criteria for evaluating diagnostic techniques. Chest **102:**553s–556s.
57. **Fagon JY, Chastre J, Hance AJ, Montravers P, Novara A, Gibert C.** 1993. Nosocomial pneumonia in ventilated patients: a cohort study evaluating attributable mortality and hospital stay. Am J Med **94:**281–288. http://dx.doi.org/10.1016/0002-9343(93)90060-3.
58. **Restrepo MI, Peterson J, Fernandez JF, Qin Z, Fisher AC, Nicholson SC.** 2013. Comparison of the bacterial etiology of early-onset and late-onset ventilator-associated pneumonia in subjects enrolled in 2 large clinical studies. Respir Care **58:**1220–1225. http://dx.doi.org/10.4187/respcare.02173.
59. **Lee MS, Walker V, Chen LF, Sexton DJ, Anderson DJ.** 2013. The epidemiology of ventilator-associated pneumonia in a network of community hospitals: a prospective multicenter study. Infect Control Hosp Epidemiol **34:**657–662. http://dx.doi.org/10.1086/670991.
60. **Jones RN.** 2010. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clin Infect Dis **51:**S81–S87. http://dx.doi.org/10.1086/653053.
61. **Paganin F, Lilienthal F, Bourdin A, Lugagne N, Tixier F, Genin R, Yvin JL.** 2004. Severe community-acquired pneumonia: assessment of microbial aetiology as mortality factor. Eur Respir J **24:**779–785. http://dx.doi.org/10.1183/09031936.04.00119503.
62. **Song JH, Oh WS, Kang CI, Chung DR, Peck KR, Ko KS, Yeom JS, Kim CK, Kim SW, Chang HH, Kim YS, Jung SI, Tong Z, Wang Q, Huang SG, Liu JW, Lalitha MK, Tan BH, Van PH, Carlos CC, So T.** 2008. Epidemiology and clinical outcomes of community-acquired pneumonia in adult patients in Asian countries: a prospective study by the Asian Network for Surveillance of Resistant Pathogens. Int J Antimicrob Agents **31:**107–114. http://dx.doi.org/10.1016/j.ijantimicag.2007.09.014.
63. **Kawai S, Ochi M, Nakagawa T, Goto H.** 2004. Antimicrobial therapy in community-acquired pneumonia among emergency patients in a university hospital in Japan. J Infect Chemother **10:**352–358. http://dx.doi.org/10.1007/s10156-004-0350-2.
64. **Feldman C, Ross S, Mahomed AG, Omar J, Smith C.** 1995. The aetiology of severe community-acquired pneumonia and its impact on initial, empiric, antimicrobial chemotherapy. Respir Med **89:**187–192. http://dx.doi.org/10.1016/0954-6111(95)90246-5.
65. **Lin YT, Jeng YY, Chen TL, Fung CP.** 2010. Bacteremic community-acquired pneumonia due to Klebsiella pneumoniae: clinical and microbiological characteristics in Taiwan, 2001-2008. BMC Infect Dis **10:**307. http://dx.doi.org/10.1186/1471-2334-10-307.
66. **Hu HC, Huang CC, Tsai YH, Lee CH, Hsieh MJ.** 2005. Outcome analysis of patients requiring mechanical ventilation with severe community-acquired pneumonia and identified bacterial pathogens. Chang Gung Med J **28:**229–236.
67. **Blue DE, Allen SD.** 2011. Microbiology for the surgical pathologist, p 315–406. *In* Cheng L, Bostwick GD (ed), Essentials of anatomic pathology. Springer, New York, NY. http://dx.doi.org/10.1007/978-1-4419-6043-6_6.
68. **Moon WK, Im JG, Yeon KM, Han MC.** 1995. Complications of Klebsiella pneumonia: CT evaluation. J Comput Assist Tomogr **19:**176–181. http://dx.doi.org/10.1097/00004728-199503000-00002.
69. **Czaja CA, Scholes D, Hooton TM, Stamm WE.** 2007. Population-based epidemiologic analysis of acute pyelonephritis. Clin Infect Dis **45:**273–280. http://dx.doi.org/10.1086/519268.
70. **Echols RM, Tosiello RL, Haverstock DC, Tice AD.** 1999. Demographic, clinical, and treatment parameters influencing the outcome of acute cystitis. Clin Infect Dis **29:**113–119. http://dx.doi.org/10.1086/520138.
71. **Lorente Garin JA, Placer Santos J, Salvado Costa M, Segura Alvarez C, Gelabert-Mas A.** 2005. Antibiotic resistance transformation in community-acquired urinary infections. Rev Clin Esp **205:**259–264. (In Spanish.) http://dx.doi.org/10.1157/13076148.
72. **Laupland KB, Ross T, Pitout JD, Church DL, Gregson DB.** 2007. Community-onset urinary tract infections: a population-based assessment. Infection **35:**150–153. http://dx.doi.org/10.1007/s15010-007-6180-2.
73. **Linhares I, Raposo T, Rodrigues A, Almeida A.** 2013. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000-2009). BMC Infect Dis **13:**19. http://dx.doi.org/10.1186/1471-2334-13-19.
74. **Rodriguez-Bano J, Lopez-Cerero L, Navarro MD, Diaz de Alba P, Pascual A.** 2008. Faecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli: prevalence, risk factors and molecular epidemiology. J Antimicrob Chemother **62:**1142–1149. http://dx.doi.org/10.1093/jac/dkn293.
75. **Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME.** 2008. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev **21:**26–59. http://dx.doi.org/10.1128/CMR.00019-07.
76. **Wu YH, Chen PL, Hung YP, Ko WC.** 2014. Risk factors and clinical impact of levofloxacin or cefazolin nonsusceptibility or ESBL production among uropathogens in adults with community-onset urinary tract infections. J Microbiol Immunol Infect **47:**197–203. http://dx.doi.org/10.1016/j.jmii.2012.09.001.
77. **Tsai SS, Huang JC, Chen ST, Sun JH, Wang CC, Lin SF, Hsu BR, Lin JD, Huang SY, Huang YY.** 2010. Characteristics of Klebsiella pneumoniae bacteremia in community-acquired and nosocomial infections in diabetic patients. Chang Gung Med J **33:**532–539.
78. **Ortega M, Marco F, Soriano A, Almela M, Martinez JA, Pitart C, Mensa J.** 2013. Epidemiology and prognostic determinants of bacteraemic catheter-acquired urinary tract infection in a single institution from 1991 to 2010. J Infect **67:**282–287. http://dx.doi.org/10.1016/j.jinf.2013.06.003.
79. **Podschun R, Ullmann U.** 1998. Klebsiella spp. as nosocomial patho-

gens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev **11:**589–603.
80. **Chetcuti Zammit S, Azzopardi N, Sant J.** 2014. Mortality risk score for Klebsiella pneumoniae bacteraemia. Eur J Intern Med **25:**571–576. http://dx.doi.org/10.1016/j.ejim.2014.04.008.
81. **Marra AR, Wey SB, Castelo A, Gales AC, Cal RG, Filho JR, Edmond MB, Pereira CA.** 2006. Nosocomial bloodstream infections caused by Klebsiella pneumoniae: impact of extended-spectrum beta-lactamase (ESBL) production on clinical outcome in a hospital with high ESBL prevalence. BMC Infect Dis **6:**24. http://dx.doi.org/10.1186/1471-2334-6-24.
82. **Mosqueda-Gomez JL, Montano-Loza A, Rolon AL, Cervantes C, Bobadilla-del-Valle JM, Silva-Sanchez J, Garza-Ramos U, Villasis-Keever A, Galindo-Fraga A, Palacios GM, Ponce-de-Leon A, Sifuentes-Osornio J.** 2008. Molecular epidemiology and risk factors of bloodstream infections caused by extended-spectrum beta-lactamase-producing Klebsiella pneumoniae. A case-control study. Int J Infect Dis **12:**653–659. http://dx.doi.org/10.1016/j.ijid.2008.03.008.
83. **Pena C, Pujol M, Ardanuy C, Ricart A, Pallares R, Linares J, Ariza J, Gudiol F.** 2001. An outbreak of hospital-acquired Klebsiella pneumoniae bacteraemia, including strains producing extended-spectrum beta-lactamase. J Hosp Infect **47:**53–59. http://dx.doi.org/10.1053/jhin.2000.0862.
84. **Jong GM, Hsiue TR, Chen CR, Chang HY, Chen CW.** 1995. Rapidly fatal outcome of bacteremic Klebsiella pneumoniae pneumonia in alcoholics. Chest **107:**214–217. http://dx.doi.org/10.1378/chest.107.1.214.
85. **Bilinski J, Grzesiowski P, Muszynski J, Wroblewska M, Madry K, Robak K, Dzieciatkowski T, Wiktor-Jedrzejczak W, Basak GW.** 9 March 2016. Fecal microbiota transplantation inhibits multidrug-resistant gut pathogens: preliminary report performed in an immunocompromised host. Arch Immunol Ther Exp (Warsz) http://dx.doi.org/10.1007/s00005-016-0387-9.
86. **Lagier JC, Million M, Fournier PE, Brouqui P, Raoult D.** 2015. Faecal microbiota transplantation for stool decolonization of OXA-48 carbapenemase-producing Klebsiella pneumoniae. J Hosp Infect **90:**173–174. http://dx.doi.org/10.1016/j.jhin.2015.02.013.
87. **Kim JK, Chung DR, Wie SH, Yoo JH, Park SW.** 2009. Risk factor analysis of invasive liver abscess caused by the K1 serotype Klebsiella pneumoniae. Eur J Clin Microbiol Infect Dis **28:**109–111. http://dx.doi.org/10.1007/s10096-008-0595-2.
88. **Wang JH, Liu YC, Lee SS, Yen MY, Chen YS, Wang JH, Wann SR, Lin HH.** 1998. Primary liver abscess due to Klebsiella pneumoniae in Taiwan. Clin Infect Dis **26:**1434–1438. http://dx.doi.org/10.1086/516369.
89. **Fang CT, Lai SY, Yi WC, Hsueh PR, Liu KL, Chang SC.** 2007. Klebsiella pneumoniae genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin Infect Dis **45:**284–293. http://dx.doi.org/10.1086/519262.
90. **Li B, Zhao Y, Liu C, Chen Z, Zhou D.** 2014. Molecular pathogenesis of Klebsiella pneumoniae. Future Microbiol **9:**1071–1081. http://dx.doi.org/10.2217/fmb.14.48.
91. **Keller JJ, Tsai MC, Lin CC, Lin YC, Lin HC.** 2013. Risk of infections subsequent to pyogenic liver abscess: a nationwide population-based study. Clin Microbiol Infect **19:**717–722. http://dx.doi.org/10.1111/1469-0691.12027.
92. **Tsai MH, Lu CH, Huang CR, Chuang YC, Tsai NW, Tsai HH, Chen SF, Li CS, Chang HW, Chien CC, Chang WN.** 2006. Bacterial meningitis in young adults in southern Taiwan: clinical characteristics and therapeutic outcomes. Infection **34:**2–8. http://dx.doi.org/10.1007/s15010-006-4144-6.
93. **Lu CH, Chang WN, Chuang YC, Chang HW.** 1999. Gram-negative bacillary meningitis in adult post-neurosurgical patients. Surg Neurol **52:**438–443, discussion 443–434. http://dx.doi.org/10.1016/S0090-3019(99)00129-9.
94. **Huang TT, Tseng FY, Liu TC, Hsu CJ, Chen YS.** 2005. Deep neck infection in diabetic patients: comparison of clinical picture and outcomes with nondiabetic patients. Otolaryngol Head Neck Surg **132:**943–947. http://dx.doi.org/10.1016/j.otohns.2005.01.035.
95. **Cheng NC, Tai HC, Chang SC, Chang CH, Lai HS.** 2015. Necrotizing fasciitis in patients with diabetes mellitus: clinical characteristics and risk factors for mortality. BMC Infect Dis **15:**417. http://dx.doi.org/10.1186/s12879-015-1144-0.
96. **Scheld WM.** 1991. Developments in the pathogenesis, diagnosis and treatment of nosocomial pneumonia. Surg Gynecol Obstet **172**(Suppl)**:**42–53.
97. **Hu X, Lee JS, Pianosi PT, Ryu JH.** 2015. Aspiration-related pulmonary syndromes. Chest **147:**815–823. http://dx.doi.org/10.1378/chest.14-1049.
98. **Wunderink RG, Waterer GW.** 2004. Community-acquired pneumonia: pathophysiology and host factors with focus on possible new approaches to management of lower respiratory tract infections. Infect Dis Clin North Am **18:**743–759. http://dx.doi.org/10.1016/j.idc.2004.07.004.
99. **Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM, Jr, Musher DM, Niederman MS, Torres A, Whitney CG.** 2007. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis **44**(Suppl 2)**:**S27–S72. http://dx.doi.org/10.1086/511159.
100. **Rammaert B, Goyet S, Beaute J, Hem S, Te V, Try PL, Mayaud C, Borand L, Buchy P, Guillard B, Vong S.** 2012. Klebsiella pneumoniae related community-acquired acute lower respiratory infections in Cambodia: clinical characteristics and treatment. BMC Infect Dis **12:**3. http://dx.doi.org/10.1186/1471-2334-12-3.
101. **Song JH, Thamlikitkul V, Hsueh PR.** 2011. Clinical and economic burden of community-acquired pneumonia amongst adults in the Asia-Pacific region. Int J Antimicrob Agents **38:**108–117. http://dx.doi.org/10.1016/j.ijantimicag.2011.02.017.
102. **Saibal MA, Rahman SH, Nishat L, Sikder NH, Begum SA, Islam MJ, Uddin KN.** 2012. Community acquired pneumonia in diabetic and non-diabetic hospitalized patients: presentation, causative pathogens and outcome. Bangladesh Med Res Counc Bull **38:**98–103.
103. **Zhang P, Nelson S, Summer WR, Spitzer JA.** 1997. Acute ethanol intoxication suppresses the pulmonary inflammatory response in rats challenged with intrapulmonary endotoxin. Alcohol Clin Exp Res **21:**773–778. http://dx.doi.org/10.1111/j.1530-0277.1997.tb03838.x.
104. **Quinton LJ, Nelson S, Zhang P, Happel KI, Gamble L, Bagby GJ.** 2005. Effects of systemic and local CXC chemokine administration on the ethanol-induced suppression of pulmonary neutrophil recruitment. Alcohol Clin Exp Res **29:**1198–1205. http://dx.doi.org/10.1097/01.ALC.0000171927.66130.AA.
105. **Nelson S, Summer W, Bagby G, Nakamura C, Stewart L, Lipscomb G, Andresen J.** 1991. Granulocyte colony-stimulating factor enhances pulmonary host defenses in normal and ethanol-treated rats. J Infect Dis **164:**901–906. http://dx.doi.org/10.1093/infdis/164.5.901.
106. **Happel KI, Odden AR, Zhang P, Shellito JE, Bagby GJ, Nelson S.** 2006. Acute alcohol intoxication suppresses the interleukin 23 response to Klebsiella pneumoniae infection. Alcohol Clin Exp Res **30:**1200–1207. http://dx.doi.org/10.1111/j.1530-0277.2006.00144.x.
107. **Szabo G, Verma B, Catalano D.** 1993. Selective inhibition of antigen-specific T lymphocyte proliferation by acute ethanol exposure: the role of impaired monocyte antigen presentation capacity and mediator production. J Leukoc Biol **54:**534–544.
108. **Higdon ML, Higdon JA.** 2006. Treatment of oncologic emergencies. Am Fam Physician **74:**1873–1880.
109. **Alba-Loureiro TC, Munhoz CD, Martins JO, Cerchiaro GA, Scavone C, Curi R, Sannomiya P.** 2007. Neutrophil function and metabolism in individuals with diabetes mellitus. Braz J Med Biol Res **40:**1037–1044. http://dx.doi.org/10.1590/S0100-879X2006005000143.
110. **Hodgson K, Morris J, Bridson T, Govan B, Rush C, Ketheesan N.** 2015. Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections. Immunology **144:**171–185. http://dx.doi.org/10.1111/imm.12394.
111. **Collado MC, Cernada M, Neu J, Perez-Martinez G, Gormaz M, Vento M.** 2015. Factors influencing gastrointestinal tract and microbiota immune interaction in preterm infants. Pediatr Res **77:**726–731. http://dx.doi.org/10.1038/pr.2015.54.
112. **Afroza S.** 2006. Neonatal sepsis—a global problem: an overview. Mymensingh Med J **15:**108–114.
113. **Boyd AR, Orihuela CJ.** 2011. Dysregulated inflammation as a risk factor for pneumonia in the elderly. Aging Dis **2:**487–500.
114. **Jeong SJ, Yoon SS, Han SH, Yong DE, Kim CO, Kim JM.** 2014. Evaluation of humoral immune response to nosocomial pathogen and functional status in elderly patients with sepsis. Arch Gerontol Geriatr **58:**10–14. http://dx.doi.org/10.1016/j.archger.2013.07.001.
115. **Teramoto S, Yoshida K, Hizawa N.** 2015. Update on the pathogenesis and management of pneumonia in the elderly—roles of aspiration pneu-

monia. Respir Investig **53:**178–184. http://dx.doi.org/10.1016/j.resinv.2015.01.003.
116. **Struve C, Bojer M, Krogfelt KA.** 2008. Characterization of Klebsiella pneumoniae type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence. Infect Immun **76:**4055–4065. http://dx.doi.org/10.1128/IAI.00494-08.
117. **Rosen DA, Pinkner JS, Walker JN, Elam JS, Jones JM, Hultgren SJ.** 2008. Molecular variations in Klebsiella pneumoniae and Escherichia coli FimH affect function and pathogenesis in the urinary tract. Infect Immun **76:**3346–3356. http://dx.doi.org/10.1128/IAI.00340-08.
118. **Guh AY, Bulens SN, Mu Y, Jacob JT, Reno J, Scott J, Wilson LE, Vaeth E, Lynfield R, Shaw KM, Vagnone PM, Bamberg WM, Janelle SJ, Dumyati G, Concannon C, Beldavs Z, Cunningham M, Cassidy PM, Phipps EC, Kenslow N, Travis T, Lonsway D, Rasheed JK, Limbago BM, Kallen AJ.** 2015. Epidemiology of carbapenem-resistant Enterobacteriaceae in 7 US communities, 2012-2013. JAMA **314:**1479–1487. http://dx.doi.org/10.1001/jama.2015.12480.
119. **Mishra SK, Basukala P, Basukala O, Parajuli K, Pokhrel BM, Rijal BP.** 2015. Detection of biofilm production and antibiotic resistance pattern in clinical isolates from indwelling medical devices. Curr Microbiol **70:**128–134. http://dx.doi.org/10.1007/s00284-014-0694-5.
120. **Zolfaghari PS, Wyncoll DL.** 2011. The tracheal tube: gateway to ventilator-associated pneumonia. Crit Care **15:**310. http://dx.doi.org/10.1186/cc10352.
121. **Chatterjee S, Maiti P, Dey R, Kundu A, Dey R.** 2014. Biofilms on indwelling urologic devices: microbes and antimicrobial management prospect. Ann Med Health Sci Res **4:**100–104. http://dx.doi.org/10.4103/2141-9248.126612.
122. **Fung CP, Lin YT, Lin JC, Chen TL, Yeh KM, Chang FY, Chuang HC, Wu HS, Tseng CP, Siu LK.** 2012. Klebsiella pneumoniae in gastrointestinal tract and pyogenic liver abscess. Emerg Infect Dis **18:**1322–1325. http://dx.doi.org/10.3201/eid1808.111053.
123. **Gupta A.** 2002. Hospital-acquired infections in the neonatal intensive care unit—Klebsiella pneumoniae. Semin Perinatol **26:**340–345. http://dx.doi.org/10.1053/sper.2002.36267.
124. **Papakonstantinou I, Angelopoulos E, Baraboutis I, Perivolioti E, Parisi M, Psaroudaki Z, Kampisiouli E, Argyropoulou A, Nanas S, Routsi C.** 2015. Risk factors for tracheobronchial acquisition of resistant Gram-negative bacterial pathogens in mechanically ventilated ICU patients. J Chemother **27:**283–289. http://dx.doi.org/10.1179/1973947814Y.0000000199.
125. **Haverkorn ML, Michel MF.** 1979. Nosocomial klebsiellas. I. Colonization of hospitalized patients. J Hyg (Lond) **82:**177–193.
126. **Taur Y, Pamer EG.** 2013. The intestinal microbiota and susceptibility to infection in immunocompromised patients. Curr Opin Infect Dis **26:**332–337. http://dx.doi.org/10.1097/QCO.0b013e3283630dd3.
127. **Selden R, Lee S, Wang WL, Bennett JV, Eickhoff TC.** 1971. Nosocomial Klebsiella infections: intestinal colonization as a reservoir. Ann Intern Med **74:**657–664. http://dx.doi.org/10.7326/0003-4819-74-5-657.
128. **Nguyen ML, Toye B, Kanji S, Zvonar R.** 2015. Risk factors for and outcomes of bacteremia caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species at a Canadian tertiary care hospital. Can J Hosp Pharm **68:**136–143.
129. **Gagliotti C, Giordani S, Ciccarese V, Barozzi A, Giovinazzi A, Pietrantonio AM, Moro ML, Pinelli G, Sarti M.** 2014. Risk factors for colonization with carbapenemase-producing Klebsiella pneumoniae in hospital: a matched case-control study. Am J Infect Control **42:**1006–1008. http://dx.doi.org/10.1016/j.ajic.2014.05.028.
130. **Papadimitriou-Olivgeris M, Marangos M, Christofidou M, Fligou F, Bartzavali C, Panteli ES, Vamvakopoulou S, Filos KS, Anastassiou ED.** 2014. Risk factors for infection and predictors of mortality among patients with KPC-producing Klebsiella pneumoniae bloodstream infections in the intensive care unit. Scand J Infect Dis **46:**642–648. http://dx.doi.org/10.3109/00365548.2014.923106.
131. **Kaspar T, Schweiger A, Droz S, Marschall J.** 2015. Colonization with resistant microorganisms in patients transferred from abroad: who needs to be screened? Antimicrob Resist Infect Control **4:**31. http://dx.doi.org/10.1186/s13756-015-0071-6.
132. **Zhang P, Summer WR, Bagby GJ, Nelson S.** 2000. Innate immunity and pulmonary host defense. Immunol Rev **173:**39–51. http://dx.doi.org/10.1034/j.1600-065X.2000.917306.x.
133. **Lee CH, Chang CC, Liu JW, Chen RF, Yang KD.** 2014. Sialic acid involved in hypermucoviscosity phenotype of Klebsiella pneumoniae and associated with resistance to neutrophil phagocytosis. Virulence **5:**673–679. http://dx.doi.org/10.4161/viru.32076.
134. **Kabha K, Nissimov L, Athamna A, Keisari Y, Parolis H, Parolis LA, Grue RM, Schlepper-Schafer J, Ezekowitz AR, Ohman DE, Ofek I.** 1995. Relationships among capsular structure, phagocytosis, and mouse virulence in Klebsiella pneumoniae. Infect Immun **63:**847–852.
135. **Kabha K, Schmegner J, Keisari Y, Parolis H, Schlepper-Schaeffer J, Ofek I.** 1997. SP-A enhances phagocytosis of Klebsiella by interaction with capsular polysaccharides and alveolar macrophages. Am J Physiol **272:**L344–L352.
136. **Puente JL, Finlay BB.** 2001. Pathogenic Escherichia coli, p 387–456. *In* Groisman EA (ed), Principles of bacterial pathogenesis. Academic Press, San Diego, CA. http://dx.doi.org/10.1016/B978-012304220-0/50010-8.
137. **McClane BA, Mietzner TA.** 1999. Overcoming the physical and chemical defenses of the human body, p 225–226. *In* McClane BA, Mietzner TA, Dowling JN, Phillips BA (ed), Microbial pathogenesis. Hayes Barton Press, Raleigh, NC.
138. **Ganz T.** 2002. Antimicrobial polypeptides in host defense of the respiratory tract. J Clin Invest **109:**693–697. http://dx.doi.org/10.1172/JCI0215218.
139. **Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT.** 2015. Complement system part II: role in immunity. Front Immunol **6:**257. http://dx.doi.org/10.3389/fimmu.2015.00257.
140. **Murphy K, Travers P, Walport M, Janeway C.** 2012. Janeway's immunobiology, 8th ed. Garland Science, New York, NY.
141. **Coya JM, Akinbi HT, Saenz A, Yang L, Weaver TE, Casals C.** 2015. Natural anti-infective pulmonary proteins: in vivo cooperative action of surfactant protein SP-A and the lung antimicrobial peptide SP-BN. J Immunol **195:**1628–1636. http://dx.doi.org/10.4049/jimmunol.1500778.
142. **Gross GN, Rehm SR, Pierce AK.** 1978. The effect of complement depletion on lung clearance of bacteria. J Clin Invest **62:**373–378. http://dx.doi.org/10.1172/JCI109138.
143. **Campos MA, Vargas MA, Regueiro V, Llompart CM, Alberti S, Bengoechea JA.** 2004. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun **72:**7107–7114. http://dx.doi.org/10.1128/IAI.72.12.7107-7114.2004.
144. **Albertí S, Alvarez D, Merino S, Casado MT, Vivanco F, Tomás JM, Benedí VJ.** 1996. Analysis of complement C3 deposition and degradation on Klebsiella pneumoniae. Infect Immun **64:**4726–4732.
145. **Alberti S, Marques G, Camprubi S, Merino S, Tomas JM, Vivanco F, Benedi VJ.** 1993. C1q binding and activation of the complement classical pathway by Klebsiella pneumoniae outer membrane proteins. Infect Immun **61:**852–860.
146. **Álvarez D, Merino S, Tomás JM, Benedí VJ, Albertí S.** 2000. Capsular polysaccharide is a major complement resistance factor in lipopolysaccharide O side chain-deficient Klebsiella pneumoniae clinical isolates. Infect Immun **68:**953–955. http://dx.doi.org/10.1128/IAI.68.2.953-955.2000.
147. **Ciurana B, Tomas JM.** 1987. Role of lipopolysaccharide and complement in susceptibility of Klebsiella pneumoniae to nonimmune serum. Infect Immun **55:**2741–2746.
148. **Tomas JM, Benedi VJ, Ciurana B, Jofre J.** 1986. Role of capsule and O antigen in resistance of Klebsiella pneumoniae to serum bactericidal activity. Infect Immun **54:**85–89.
149. **Moranta D, Regueiro V, March C, Llobet E, Margareto J, Larrarte E, Garmendia J, Bengoechea JA.** 2010. Klebsiella pneumoniae capsule polysaccharide impedes the expression of beta-defensins by airway epithelial cells. Infect Immun **78:**1135–1146. http://dx.doi.org/10.1128/IAI.00940-09.
150. **Kostina E, Ofek I, Crouch E, Friedman R, Sirota L, Klinger G, Sahly H, Keisari Y.** 2005. Noncapsulated Klebsiella pneumoniae bearing mannose-containing O antigens is rapidly eradicated from mouse lung and triggers cytokine production by macrophages following opsonization with surfactant protein D. Infect Immun **73:**8282–8290. http://dx.doi.org/10.1128/IAI.73.12.8282-8290.2005.
151. **Sahly H, Schubert S, Harder J, Kleine M, Sandvang D, Ullmann U, Schroder JM, Podschun R.** 2006. Activity of human beta-defensins 2 and 3 against ESBL-producing Klebsiella strains. J Antimicrob Chemother **57:**562–565. http://dx.doi.org/10.1093/jac/dkl003.
152. **Clements A, Tull D, Jenney AW, Farn JL, Kim SH, Bishop RE, McPhee JB, Hancock RE, Hartland EL, Pearse MJ, Wijburg OL, Jackson DC, McConville MJ, Strugnell RA.** 2007. Secondary acylation of Klebsiella

pneumoniae lipopolysaccharide contributes to sensitivity to antibacterial peptides. J Biol Chem **282:**15569–15577. http://dx.doi.org/10.1074/jbc.M701454200.
153. **Broug-Holub E, Toews GB, van Iwaarden JF, Strieter RM, Kunkel SL, Paine R, III, Standiford TJ.** 1997. Alveolar macrophages are required for protective pulmonary defenses in murine Klebsiella pneumonia: elimination of alveolar macrophages increases neutrophil recruitment but decreases bacterial clearance and survival. Infect Immun **65:**1139–1146.
154. **Kumar V, Sharma A.** 2010. Neutrophils: Cinderella of innate immune system. Int Immunopharmacol **10:**1325–1334. http://dx.doi.org/10.1016/j.intimp.2010.08.012.
155. **Batra S, Cai S, Balamayooran G, Jeyaseelan S.** 2012. Intrapulmonary administration of leukotriene B(4) augments neutrophil accumulation and responses in the lung to Klebsiella infection in CXCL1 knockout mice. J Immunol **188:**3458–3468. http://dx.doi.org/10.4049/jimmunol.1101985.
156. **Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, Schwarzenberger P, Shellito JE, Kolls JK.** 2001. Interleukin-17 and lung host defense against Klebsiella pneumoniae infection. Am J Respir Cell Mol Biol **25:**335–340. http://dx.doi.org/10.1165/ajrcmb.25.3.4424.
157. **Belaaouaj A, McCarthy R, Baumann M, Gao Z, Ley TJ, Abraham SN, Shapiro SD.** 1998. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. Nat Med **4:**615–618. http://dx.doi.org/10.1038/nm0598-615.
158. **Hirche TO, Gaut JP, Heinecke JW, Belaaouaj A.** 2005. Myeloperoxidase plays critical roles in killing Klebsiella pneumoniae and inactivating neutrophil elastase: effects on host defense. J Immunol **174:**1557–1565. http://dx.doi.org/10.4049/jimmunol.174.3.1557.
159. **Greenberg SS, Ouyang J, Zhao X, Parrish C, Nelson S, Giles TD.** 1999. Effects of ethanol on neutrophil recruitment and lung host defense in nitric oxide synthase I and nitric oxide synthase II knockout mice. Alcohol Clin Exp Res **23:**1435–1445. http://dx.doi.org/10.1111/j.1530-0277.1999.tb04664.x.
160. **Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, Papayannopoulos V.** 2014. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nat Immunol **15:**1017–1025. http://dx.doi.org/10.1038/ni.2987.
161. **Sharma A, Steichen AL, Jondle CN, Mishra BB, Sharma J.** 2014. Protective role of Mincle in bacterial pneumonia by regulation of neutrophil mediated phagocytosis and extracellular trap formation. J Infect Dis **209:**1837–1846. http://dx.doi.org/10.1093/infdis/jit820.
162. **Barletta KE, Cagnina RE, Burdick MD, Linden J, Mehrad B.** 2012. Adenosine A(2B) receptor deficiency promotes host defenses against gram-negative bacterial pneumonia. Am J Respir Crit Care Med **186:**1044–1050. http://dx.doi.org/10.1164/rccm.201204-0622OC.
163. **Achouiti A, Vogl T, Urban CF, Rohm M, Hommes TJ, van Zoelen MA, Florquin S, Roth J, van't Veer C, de Vos AF, van der Poll T.** 2012. Myeloid-related protein-14 contributes to protective immunity in gram-negative pneumonia derived sepsis. PLoS Pathog **8:**e1002987. http://dx.doi.org/10.1371/journal.ppat.1002987.
164. **Ariffin H, Navaratnam P, Lin HP.** 2002. Surveillance study of bacteraemic episodes in febrile neutropenic children. Int J Clin Pract **56:**237–240.
165. **Vento S, Cainelli F.** 2003. Infections in patients with cancer undergoing chemotherapy: aetiology, prevention, and treatment. Lancet Oncol **4:**595–604. http://dx.doi.org/10.1016/S1470-2045(03)01218-X.
166. **Velasco E, Byington R, Martins CA, Schirmer M, Dias LM, Goncalves VM.** 2006. Comparative study of clinical characteristics of neutropenic and non-neutropenic adult cancer patients with bloodstream infections. Eur J Clin Microbiol Infect Dis **25:**1–7. http://dx.doi.org/10.1007/s10096-005-0077-8.
167. **Lawlor MS, Hsu J, Rick PD, Miller VL.** 2005. Identification of Klebsiella pneumoniae virulence determinants using an intranasal infection model. Mol Microbiol **58:**1054–1073. http://dx.doi.org/10.1111/j.1365-2958.2005.04918.x.
168. **Lau HY, Clegg S, Moore TA.** 2007. Identification of Klebsiella pneumoniae genes uniquely expressed in a strain virulent using a murine model of bacterial pneumonia. Microb Pathog **42:**148–155. http://dx.doi.org/10.1016/j.micpath.2007.01.001.
169. **Tsai WC, Strieter RM, Wilkowski JM, Bucknell KA, Burdick MD, Lira SA, Standiford TJ.** 1998. Lung-specific transgenic expression of KC enhances resistance to Klebsiella pneumoniae in mice. J Immunol **161:**2435–2440.
170. **Cai S, Batra S, Shen L, Wakamatsu N, Jeyaseelan S.** 2009. Both TRIF- and MyD88-dependent signaling contribute to host defense against pulmonary Klebsiella infection. J Immunol **183:**6629–6638. http://dx.doi.org/10.4049/jimmunol.0901033.
171. **Chen L, Zhang Z, Barletta KE, Burdick MD, Mehrad B.** 2013. Heterogeneity of lung mononuclear phagocytes during pneumonia: contribution of chemokine receptors. Am J Physiol Lung Cell Mol Physiol **305:**L702–L711. http://dx.doi.org/10.1152/ajplung.00194.2013.
172. **Bhan U, Lukacs NW, Osterholzer JJ, Newstead MW, Zeng X, Moore TA, McMillan TR, Krieg AM, Akira S, Standiford TJ.** 2007. TLR9 is required for protective innate immunity in Gram-negative bacterial pneumonia: role of dendritic cells. J Immunol **179:**3937–3946. http://dx.doi.org/10.4049/jimmunol.179.6.3937.
173. **Schurr JR, Young E, Byrne P, Steele C, Shellito JE, Kolls JK.** 2005. Central role of Toll-like receptor 4 signaling and host defense in experimental pneumonia caused by Gram-negative bacteria. Infect Immun **73:**532–545. http://dx.doi.org/10.1128/IAI.73.1.532-545.2005.
174. **Jeyaseelan S, Young SK, Yamamoto M, Arndt PG, Akira S, Kolls JK, Worthen GS.** 2006. Toll/IL-1R domain-containing adaptor protein (TIRAP) is a critical mediator of antibacterial defense in the lung against Klebsiella pneumoniae but not Pseudomonas aeruginosa. J Immunol **177:**538–547. http://dx.doi.org/10.4049/jimmunol.177.1.538.
175. **van Lieshout MH, Blok DC, Wieland CW, de Vos AF, van't Veer C, van der Poll T.** 2012. Differential roles of MyD88 and TRIF in hematopoietic and resident cells during murine gram-negative pneumonia. J Infect Dis **206:**1415–1423. http://dx.doi.org/10.1093/infdis/jis505.
176. **Wieland CW, van Lieshout MH, Hoogendijk AJ, van der Poll T.** 2011. Host defence during Klebsiella pneumonia relies on haematopoietic-expressed Toll-like receptors 4 and 2. Eur Respir J **37:**848–857. http://dx.doi.org/10.1183/09031936.00076510.
177. **Bhan U, Ballinger MN, Zeng X, Newstead MJ, Cornicelli MD, Standiford TJ.** 2010. Cooperative interactions between TLR4 and TLR9 regulate interleukin 23 and 17 production in a murine model of gram negative bacterial pneumonia. PLoS One **5:**e9896. http://dx.doi.org/10.1371/journal.pone.0009896.
178. **Standiford LR, Standiford TJ, Newstead MJ, Zeng X, Ballinger MN, Kovach MA, Reka AK, Bhan U.** 2012. TLR4-dependent GM-CSF protects against lung injury in Gram-negative bacterial pneumonia. Am J Physiol Lung Cell Mol Physiol **302:**L447–L454. http://dx.doi.org/10.1152/ajplung.00415.2010.
179. **Happel KI, Zheng M, Young E, Quinton LJ, Lockhart E, Ramsay AJ, Shellito JE, Schurr JR, Bagby GJ, Nelson S, Kolls JK.** 2003. Cutting edge: roles of Toll-like receptor 4 and IL-23 in IL-17 expression in response to Klebsiella pneumoniae infection. J Immunol **170:**4432–4436. http://dx.doi.org/10.4049/jimmunol.170.9.4432.
180. **Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, Odden AR, Shellito JE, Bagby GJ, Nelson S, Kolls JK.** 2005. Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. J Exp Med **202:**761–769. http://dx.doi.org/10.1084/jem.20050193.
181. **Price AE, Reinhardt RL, Liang HE, Locksley RM.** 2012. Marking and quantifying IL-17A-producing cells in vivo. PLoS One **7:**e39750. http://dx.doi.org/10.1371/journal.pone.0039750.
182. **Cai S, Batra S, Wakamatsu N, Pacher P, Jeyaseelan S.** 2012. NLRC4 inflammasome-mediated production of IL-1beta modulates mucosal immunity in the lung against gram-negative bacterial infection. J Immunol **188:**5623–5635. http://dx.doi.org/10.4049/jimmunol.1200195.
183. **Moore TA, Moore BB, Newstead MW, Standiford TJ.** 2000. Gamma delta-T cells are critical for survival and early proinflammatory cytokine gene expression during murine Klebsiella pneumonia. J Immunol **165:**2643–2650. http://dx.doi.org/10.4049/jimmunol.165.5.2643.
184. **Cogen AL, Moore TA.** 2009. Beta2-microglobulin-dependent bacterial clearance and survival during murine Klebsiella pneumoniae bacteremia. Infect Immun **77:**360–366. http://dx.doi.org/10.1128/IAI.00909-08.
185. **Moore TA, Perry ML, Getsoian AG, Newstead MW, Standiford TJ.** 2002. Divergent role of gamma interferon in a murine model of pulmonary versus systemic Klebsiella pneumoniae infection. Infect Immun **70:**6310–6318. http://dx.doi.org/10.1128/IAI.70.11.6310-6318.2002.
186. **Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Iwakurai Y, Yamaguchi K.** 2001. Protection against pulmonary infection with Klebsiella pneumoniae in mice by interferon-gamma through activation

of phagocytic cells and stimulation of production of other cytokines. J Med Microbiol **50:**959–964. http://dx.doi.org/10.1099/0022-1317-50-11-959.

187. **Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, Husain S, Kreindler JL, Dubin PJ, Pilewski JM, Myerburg MM, Mason CA, Iwakura Y, Kolls JK.** 2008. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med **14:**275–281. http://dx.doi.org/10.1038/nm1710.
188. **Zheng M, Horne W, McAleer JP, Pociask D, Eddens T, Good M, Gao B, Kolls JK.** 2016. Therapeutic role of interleukin 22 in experimental intra-abdominal Klebsiella pneumoniae infection in mice. Infect Immun **84:**782–789. http://dx.doi.org/10.1128/IAI.01268-15.
189. **Xu X, Weiss ID, Zhang HH, Singh SP, Wynn TA, Wilson MS, Farber JM.** 2014. Conventional NK cells can produce IL-22 and promote host defense in Klebsiella pneumoniae pneumonia. J Immunol **192:**1778–1786. http://dx.doi.org/10.4049/jimmunol.1300039.
190. **Georgel P, Radosavljevic M, Macquin C, Bahram S.** 2011. The non-conventional MHC class I MR1 molecule controls infection by Klebsiella pneumoniae in mice. Mol Immunol **48:**769–775. http://dx.doi.org/10.1016/j.molimm.2010.12.002.
191. **Ussher JE, Klenerman P, Willberg CB.** 2014. Mucosal-associated invariant T-cells: new players in anti-bacterial immunity. Front Immunol **5:**450. http://dx.doi.org/10.3389/fimmu.2014.00450.
192. **Willingham SB, Allen IC, Bergstralh DT, Brickey WJ, Huang MT, Taxman DJ, Duncan JA, Ting JP.** 2009. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. J Immunol **183:**2008–2015. http://dx.doi.org/10.4049/jimmunol.0900138.
193. **Webster SJ, Daigneault M, Bewley MA, Preston JA, Marriott HM, Walmsley SR, Read RC, Whyte MK, Dockrell DH.** 2010. Distinct cell death programs in monocytes regulate innate responses following challenge with common causes of invasive bacterial disease. J Immunol **185:**2968–2979. http://dx.doi.org/10.4049/jimmunol.1000805.
194. **Cortes G, Borrell N, de Astorza B, Gomez C, Sauleda J, Alberti S.** 2002. Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of Klebsiella pneumoniae in a murine model of pneumonia. Infect Immun **70:**2583–2590. http://dx.doi.org/10.1128/IAI.70.5.2583-2590.2002.
195. **Trosky JE, Liverman AD, Orth K.** 2008. Yersinia outer proteins: Yops. Cell Microbiol **10:**557–565. http://dx.doi.org/10.1111/j.1462-5822.2007.01109.x.
196. **Domenico P, Salo RJ, Cross AS, Cunha BA.** 1994. Polysaccharide capsule-mediated resistance to opsonophagocytosis in Klebsiella pneumoniae. Infect Immun **62:**4495–4499.
197. **Lawlor MS, Handley SA, Miller VL.** 2006. Comparison of the host responses to wild-type and cpsB mutant Klebsiella pneumoniae infections. Infect Immun **74:**5402–5407. http://dx.doi.org/10.1128/IAI.00244-06.
198. **Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Yamaguchi K.** 2000. Role of bacterial capsule in local and systemic inflammatory responses of mice during pulmonary infection with Klebsiella pneumoniae. J Med Microbiol **49:**1003–1010. http://dx.doi.org/10.1099/0022-1317-49-11-1003.
199. **Fang C-T, Chuang Y-P, Shun C-T, Chang S-C, Wang J-T.** 2004. A novel virulence gene in Klebsiella pneumoniae strains causing primary liver abscess and septic metastatic complications. J Exp Med **199:**697–705. http://dx.doi.org/10.1084/jem.20030857.
200. **Yeh K-M, Kurup A, Siu LK, Koh YL, Fung C-P, Lin J-C, Chen T-L, Chang F-Y, Koh T-H.** 2007. Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for Klebsiella pneumoniae liver abscess in Singapore and Taiwan. J Clin Microbiol **45:**466–471. http://dx.doi.org/10.1128/JCM.01150-06.
201. **Pan Y-J, Fang H-C, Yang H-C, Lin T-L, Hsieh P-F, Tsai F-C, Keynan Y, Wang J-T.** 2008. Capsular polysaccharide synthesis regions in Klebsiella pneumoniae serotype K57 and a new capsular serotype. J Clin Microbiol **46:**2231–2240. http://dx.doi.org/10.1128/JCM.01716-07.
202. **Arakawa Y, Ohta M, Wacharotayankun R, Mori M, Kido N, Ito H, Komatsu T, Sugiyama T, Kato N.** 1991. Biosynthesis of Klebsiella K2 capsular polysaccharide in Escherichia coli HB101 requires the functions of rmpA and the chromosomal cps gene cluster of the virulent strain Klebsiella pneumoniae Chedid (O1:K2). Infect Immun **59:**2043–2050.
203. **Shu HY, Fung CP, Liu YM, Wu KM, Chen YT, Li LH, Liu TT, Kirby R, Tsai SF.** 2009. Genetic diversity of capsular polysaccharide biosynthesis in Klebsiella pneumoniae clinical isolates. Microbiology **155:**4170–4183. http://dx.doi.org/10.1099/mic.0.029017-0.
204. **Pan Y-J, Lin T-L, Chen Y-H, Hsu C-R, Hsieh P-F, Wu M-C, Wang J-T.** 2013. Capsular types of Klebsiella pneumoniae revisited by wzc sequencing. PLoS One **8:**e80670. http://dx.doi.org/10.1371/journal.pone.0080670.
205. **Arakawa Y, Wacharotayankun R, Nagatsuka T, Ito H, Kato N, Ohta M.** 1995. Genomic organization of the Klebsiella pneumoniae cps region responsible for serotype K2 capsular polysaccharide synthesis in the virulent strain Chedid. J Bacteriol **177:**1788–1796.
206. **Brisse S, Passet V, Haugaard AB, Babosan A, Kassis-Chikhani N, Struve C, Decre D.** 2013. wzi gene sequencing, a rapid method for determination of capsular type for Klebsiella strains. J Clin Microbiol **51:**4073–4078. http://dx.doi.org/10.1128/JCM.01924-13.
207. **Diago-Navarro E, Chen L, Passet V, Burack S, Ulacia-Hernando A, Kodiyanplakkal RP, Levi MH, Brisse S, Kreiswirth BN, Fries BC.** 2014. Carbapenem-resistant Klebsiella pneumoniae exhibit variability in capsular polysaccharide and capsule associated virulence traits. J Infect Dis **210:**803–813. http://dx.doi.org/10.1093/infdis/jiu157.
208. **Cubero M, Grau I, Tubau F, Pallares R, Dominguez MA, Linares J, Ardanuy C.** 2016. Hypervirulent Klebsiella pneumoniae clones causing bacteraemia in adults in a teaching hospital in Barcelona, Spain (2007-2013). Clin Microbiol Infect **22:**154–160. http://dx.doi.org/10.1016/j.cmi.2015.09.025.
209. **Park DJ, Yu JK, Park KG, Park YJ.** 2015. Genotypes of ciprofloxacin-resistant Klebsiella pneumoniae in Korea and their characteristics according to the genetic lineages. Microb Drug Resist **21:**622–630. http://dx.doi.org/10.1089/mdr.2015.0001.
210. **Gomez-Simmonds A, Greenman M, Sullivan SB, Tanner JP, Sowash MG, Whittier S, Uhlemann AC.** 2015. Population structure of Klebsiella pneumoniae causing bloodstream infections at a New York City tertiary care hospital: diversification of multidrug-resistant isolates. J Clin Microbiol **53:**2060–2067. http://dx.doi.org/10.1128/JCM.03455-14.
211. **Bushell SR, Mainprize IL, Wear MA, Lou H, Whitfield C, Naismith JH.** 2013. Wzi is an outer membrane lectin that underpins group 1 capsule assembly in Escherichia coli. Structure **21:**844–853. http://dx.doi.org/10.1016/j.str.2013.03.010.
212. **Rahn A, Beis K, Naismith JH, Whitfield C.** 2003. A novel outer membrane protein, Wzi, is involved in surface assembly of the Escherichia coli K30 group 1 capsule. J Bacteriol **185:**5882–5890. http://dx.doi.org/10.1128/JB.185.19.5882-5890.2003.
213. **Rahn A, Drummelsmith J, Whitfield C.** 1999. Conserved organization in the cps gene clusters for expression of Escherichia coli group 1 K antigens: relationship to the colanic acid biosynthesis locus and the cps genes from Klebsiella pneumoniae. J Bacteriol **181:**2307–2313.
214. **Hsu CR, Lin TL, Chen YC, Chou HC, Wang JT.** 2011. The role of Klebsiella pneumoniae rmpA in capsular polysaccharide synthesis and virulence revisited. Microbiology **157:**3446–3457. http://dx.doi.org/10.1099/mic.0.050336-0.
215. **Nassif X, Honore N, Vasselon T, Cole ST, Sansonetti PJ.** 1989. Positive control of colanic acid synthesis in Escherichia coli by rmpA and rmpB, two virulence-plasmid genes of Klebsiella pneumoniae. Mol Microbiol **3:**1349–1359. http://dx.doi.org/10.1111/j.1365-2958.1989.tb00116.x.
216. **Lai YC, Peng HL, Chang HY.** 2003. RmpA2, an activator of capsule biosynthesis in Klebsiella pneumoniae CG43, regulates K2 cps gene expression at the transcriptional level. J Bacteriol **185:**788–800. http://dx.doi.org/10.1128/JB.185.3.788-800.2003.
217. **Wacharotayankun R, Arakawa Y, Ohta M, Tanaka K, Akashi T, Mori M, Kato N.** 1993. Enhancement of extracapsular polysaccharide synthesis in Klebsiella pneumoniae by RmpA2, which shows homology to NtrC and FixJ. Infect Immun **61:**3164–3174.
218. **Allen P, Hart CA, Saunders JR.** 1987. Isolation from Klebsiella and characterization of two rcs genes that activate colanic acid capsular biosynthesis in Escherichia coli. J Gen Microbiol **133:**331–340.
219. **Stout V, Torres-Cabassa A, Maurizi MR, Gutnick D, Gottesman S.** 1991. RcsA, an unstable positive regulator of capsular polysaccharide synthesis. J Bacteriol **173:**1738–1747.
220. **Chuang Y-P, Fang C-T, Lai S-Y, Chang S-C, Wang J-T.** 2006. Genetic determinants of capsular serotype K1 of Klebsiella pneumoniae causing primary pyogenic liver abscess. J Infect Dis **193:**645–654. http://dx.doi.org/10.1086/499968.
221. **Struve C, Bojer M, Nielsen EM, Hansen DS, Krogfelt KA.** 2005. Investigation of the putative virulence gene magA in a worldwide collec-

tion of 495 Klebsiella isolates: magA is restricted to the gene cluster of Klebsiella pneumoniae capsule serotype K1. J Med Microbiol **54:**1111–1113. http://dx.doi.org/10.1099/jmm.0.46165-0.
222. **Yeh KM, Chang FY, Fung CP, Lin JC, Siu LK.** 2006. magA is not a specific virulence gene for Klebsiella pneumoniae strains causing liver abscess but is part of the capsular polysaccharide gene cluster of K. pneumoniae serotype K1. J Med Microbiol **55:**803–804. http://dx.doi.org/10.1099/jmm.0.46368-0.
223. **Fang C-T, Lai S-Y, Yi W-C, Hsueh P-R, Liu K-L.** 2010. The function of wzy_K1 (magA), the serotype K1 polymerase gene in Klebsiella pneumoniae cps gene cluster. J Infect Dis **201:**1268–1269. http://dx.doi.org/10.1086/652183.
224. **Yu W-L, Fung C-P, Ko W-C, Chuang Y-C.** 2007. Polymerase chain reaction analysis for detecting capsule serotypes K1 and K2 of Klebsiella pneumoniae causing abscesses of the liver and other sites. J Infect Dis **195:**1235–1236. http://dx.doi.org/10.1086/512686.
225. **Cryz SJ, Jr, Mortimer PM, Mansfield V, Germanier R.** 1986. Seroepidemiology of Klebsiella bacteremic isolates and implications for vaccine development. J Clin Microbiol **23:**687–690.
226. **Mizuta K, Ohta M, Mori M, Hasegawa T, Nakashima I, Kato N.** 1983. Virulence for mice of Klebsiella strains belonging to the O1 group: relationship to their capsular (K) types. Infect Immun **40:**56–61.
227. **Yu WL, Ko WC, Cheng KC, Lee CC, Lai CC, Chuang YC.** 2008. Comparison of prevalence of virulence factors for Klebsiella pneumoniae liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. Diagn Microbiol Infect Dis **62:**1–6. http://dx.doi.org/10.1016/j.diagmicrobio.2008.04.007.
228. **Sahly H, Aucken H, Benedi VJ, Forestier C, Fussing V, Hansen DS, Ofek I, Podschun R, Sirot D, Sandvang D, Tomas JM, Ullmann U.** 2004. Impairment of respiratory burst in polymorphonuclear leukocytes by extended-spectrum beta-lactamase-producing strains of Klebsiella pneumoniae. Eur J Clin Microbiol Infect Dis **23:**20–26. http://dx.doi.org/10.1007/s10096-003-1047-7.
229. **Lin JC, Chang FY, Fung CP, Xu JZ, Cheng HP, Wang JJ, Huang LY, Siu LK.** 2004. High prevalence of phagocytic-resistant capsular serotypes of Klebsiella pneumoniae in liver abscess. Microbes Infect **6:**1191–1198. http://dx.doi.org/10.1016/j.micinf.2004.06.003.
230. **Tigyi Z, Gahrs W, Emody L, Makovitzky J.** 2009. Topo-optical investigations on the surface of bacterial cells during the phagocytosis of Klebsiella pneumoniae in mouse. Acta Histochem **111:**300–307. http://dx.doi.org/10.1016/j.acthis.2008.11.009.
231. **Varki A, Schauer R.** 2009. Sialic acids, p 199–218. *In* Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME (ed), Essentials of glycobiology, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
232. **Athamna A, Ofek I, Keisari Y, Markowitz S, Dutton GG, Sharon N.** 1991. Lectinophagocytosis of encapsulated Klebsiella pneumoniae mediated by surface lectins of guinea pig alveolar macrophages and human monocyte-derived macrophages. Infect Immun **59:**1673–1682.
233. **Ofek I, Goldhar J, Keisari Y, Sharon N.** 1995. Nonopsonic phagocytosis of microorganisms. Annu Rev Microbiol **49:**239–276. http://dx.doi.org/10.1146/annurev.mi.49.100195.001323.
234. **Sahly H, Keisari Y, Crouch E, Sharon N, Ofek I.** 2008. Recognition of bacterial surface polysaccharides by lectins of the innate immune system and its contribution to defense against infection: the case of pulmonary pathogens. Infect Immun **76:**1322–1332. http://dx.doi.org/10.1128/IAI.00910-07.
235. **Struve C, Roe CC, Stegger M, Stahlhut SG, Hansen DS, Engelthaler DM, Andersen PS, Driebe EM, Keim P, Krogfelt KA.** 2015. Mapping the evolution of hypervirulent Klebsiella pneumoniae. mBio **6:**e00630–15. http://dx.doi.org/10.1128/mBio.00630-15.
236. **Luo Y, Wang Y, Ye L, Yang J.** 2014. Molecular epidemiology and virulence factors of pyogenic liver abscess causing Klebsiella pneumoniae in China. Clin Microbiol Infect **20:**O818–O824. http://dx.doi.org/10.1111/1469-0691.12664.
237. **Chung DR, Lee HR, Lee SS, Kim SW, Chang HH, Jung SI, Oh MD, Ko KS, Kang CI, Peck KR, Song JH.** 2008. Evidence for clonal dissemination of the serotype K1 Klebsiella pneumoniae strain causing invasive liver abscesses in Korea. J Clin Microbiol **46:**4061–4063. http://dx.doi.org/10.1128/JCM.01577-08.
238. **Siu LK, Fung CP, Chang FY, Lee N, Yeh KM, Koh TH, Ip M.** 2011. Molecular typing and virulence analysis of serotype K1 Klebsiella pneumoniae strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. J Clin Microbiol **49:**3761–3765. http://dx.doi.org/10.1128/JCM.00977-11.
239. **Liao CH, Huang YT, Chang CY, Hsu HS, Hsueh PR.** 2014. Capsular serotypes and multilocus sequence types of bacteremic Klebsiella pneumoniae isolates associated with different types of infections. Eur J Clin Microbiol Infect Dis **33:**365–369. http://dx.doi.org/10.1007/s10096-013-1964-z.
240. **Turton JF, Englender H, Gabriel SN, Turton SE, Kaufmann ME, Pitt TL.** 2007. Genetically similar isolates of Klebsiella pneumoniae serotype K1 causing liver abscesses in three continents. J Med Microbiol **56:**593–597. http://dx.doi.org/10.1099/jmm.0.46964-0.
241. **Decre D, Verdet C, Emirian A, Le Gourrierec T, Petit JC, Offenstadt G, Maury E, Brisse S, Arlet G.** 2011. Emerging severe and fatal infections due to Klebsiella pneumoniae in two university hospitals in France. J Clin Microbiol **49:**3012–3014. http://dx.doi.org/10.1128/JCM.00676-11.
242. **Evrard B, Balestrino D, Dosgilbert A, Bouya-Gachancard JLJ, Charbonnel N, Forestier C, Tridon A.** 2010. Roles of capsule and lipopolysaccharide O antigen in interactions of human monocyte-derived dendritic cells and Klebsiella pneumoniae. Infect Immun **78:**210–219. http://dx.doi.org/10.1128/IAI.00864-09.
243. **Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Yamaguchi K.** 2001. Induction of interleukin-10 and down-regulation of cytokine production by Klebsiella pneumoniae capsule in mice with pulmonary infection. J Med Microbiol **50:**456–461. http://dx.doi.org/10.1099/0022-1317-50-5-456.
244. **Regueiro V, Moranta D, Frank CG, Larrarte E, Margareto J, March C, Garmendia J, Bengoechea JA.** 2011. Klebsiella pneumoniae subverts the activation of inflammatory responses in a NOD1-dependent manner. Cell Microbiol **13:**135–153. http://dx.doi.org/10.1111/j.1462-5822.2010.01526.x.
245. **Clements A, Gaboriaud F, Duval JF, Farn JL, Jenney AW, Lithgow T, Wijburg OL, Hartland EL, Strugnell RA.** 2008. The major surface-associated saccharides of Klebsiella pneumoniae contribute to host cell association. PLoS One **3:**e3817. http://dx.doi.org/10.1371/journal.pone.0003817.
246. **Simoons-Smit AM, Verweij-van Vught AM, MacLaren DM.** 1986. The role of K antigens as virulence factors in Klebsiella. J Med Microbiol **21:**133–137. http://dx.doi.org/10.1099/00222615-21-2-133.
247. **Llobet E, Tomas JM, Bengoechea JA.** 2008. Capsule polysaccharide is a bacterial decoy for antimicrobial peptides. Microbiology **154:**3877–3886. http://dx.doi.org/10.1099/mic.0.2008/022301-0.
248. **Llobet E, Campos MA, Gimenez P, Moranta D, Bengoechea JA.** 2011. Analysis of the networks controlling the antimicrobial-peptide-dependent induction of Klebsiella pneumoniae virulence factors. Infect Immun **79:**3718–3732. http://dx.doi.org/10.1128/IAI.05226-11.
249. **Pomakova DK, Hsiao CB, Beanan JM, Olson R, MacDonald U, Keynan Y, Russo TA.** 2012. Clinical and phenotypic differences between classic and hypervirulent Klebsiella pneumonia: an emerging and underrecognized pathogenic variant. Eur J Clin Microbiol Infect Dis **31:**981–989. http://dx.doi.org/10.1007/s10096-011-1396-6.
250. **Yeh KM, Lin JC, Yin FY, Fung CP, Hung HC, Siu LK, Chang FY.** 2010. Revisiting the importance of virulence determinant magA and its surrounding genes in Klebsiella pneumoniae causing pyogenic liver abscesses: exact role in serotype K1 capsule formation. J Infect Dis **201:**1259–1267. http://dx.doi.org/10.1086/606010.
251. **Wu JH, Wu AM, Tsai CG, Chang XY, Tsai SF, Wu TS.** 2008. Contribution of fucose-containing capsules in Klebsiella pneumoniae to bacterial virulence in mice. Exp Biol Med (Maywood) **233:**64–70. http://dx.doi.org/10.3181/0706-RM-170.
252. **Turton JF, Perry C, Elgohari S, Hampton CV.** 2010. PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. J Med Microbiol **59:**541–547. http://dx.doi.org/10.1099/jmm.0.015198-0.
253. **De Majumdar S, Yu J, Fookes M, McAteer SP, Llobet E, Finn S, Spence S, Monaghan A, Kissenpfennig A, Ingram RJ, Bengoechea J, Gally DL, Fanning S, Elborn JS, Schneiders T.** 2015. Elucidation of the RamA regulon in Klebsiella pneumoniae reveals a role in LPS regulation. PLoS Pathog **11:**e1004627. http://dx.doi.org/10.1371/journal.ppat.1004627.
254. **Frirdich E, Whitfield C.** 2005. Lipopolysaccharide inner core oligosaccharide structure and outer membrane stability in human pathogens belonging to the Enterobacteriaceae. J Endotoxin Res **11:**133–144. http://dx.doi.org/10.1177/09680519050110030201.
255. **Merino S, Altarriba M, Izquierdo L, Nogueras MM, Regue M, Tomas**

**JM.** 2000. Cloning and sequencing of the Klebsiella pneumoniae O5 wb gene cluster and its role in pathogenesis. Infect Immun **68:**2435–2440. http://dx.doi.org/10.1128/IAI.68.5.2435-2440.2000.
256. **Raetz CR, Guan Z, Ingram BO, Six DA, Song F, Wang X, Zhao J.** 2009. Discovery of new biosynthetic pathways: the lipid A story. J Lipid Res **50**(Suppl):S103–S108. http://dx.doi.org/10.1194/jlr.R800060-JLR200.
257. **Hansen DS, Mestre F, Alberti S, Hernandez-Alles S, Alvarez D, Domenech-Sanchez A, Gil J, Merino S, Tomas JM, Benedi VJ.** 1999. Klebsiella pneumoniae lipopolysaccharide O typing: revision of prototype strains and O-group distribution among clinical isolates from different sources and countries. J Clin Microbiol **37:**56–62.
258. **Branger J, Knapp S, Weijer S, Leemans JC, Pater JM, Speelman P, Florquin S, van der Poll T.** 2004. Role of Toll-like receptor 4 in Gram-positive and Gram-negative pneumonia in mice. Infect Immun **72:**788–794. http://dx.doi.org/10.1128/IAI.72.2.788-794.2004.
259. **Wu MF, Yang CY, Lin TL, Wang JT, Yang FL, Wu SH, Hu BS, Chou TY, Tsai MD, Lin CH, Hsieh SL.** 2009. Humoral immunity against capsule polysaccharide protects the host from magA$^{+}$ Klebsiella pneumoniae-induced lethal disease by evading Toll-like receptor 4 signaling. Infect Immun **77:**615–621. http://dx.doi.org/10.1128/IAI.00931-08.
260. **Montminy SW, Khan N, McGrath S, Walkowicz MJ, Sharp F, Conlon JE, Fukase K, Kusumoto S, Sweet C, Miyake K, Akira S, Cotter RJ, Goguen JD, Lien E.** 2006. Virulence factors of Yersinia pestis are overcome by a strong lipopolysaccharide response. Nat Immunol **7:**1066–1073. http://dx.doi.org/10.1038/ni1386.
261. **Stead CM, Beasley A, Cotter RJ, Trent MS.** 2008. Deciphering the unusual acylation pattern of Helicobacter pylori lipid A. J Bacteriol **190:**7012–7021. http://dx.doi.org/10.1128/JB.00667-08.
262. **Coats SR, Jones JW, Do CT, Braham PH, Bainbridge BW, To TT, Goodlett DR, Ernst RK, Darveau RP.** 2009. Human Toll-like receptor 4 responses to P. gingivalis are regulated by lipid A 1- and 4′-phosphatase activities. Cell Microbiol **11:**1587–1599. http://dx.doi.org/10.1111/j.1462-5822.2009.01349.x.
263. **Llobet E, Martínez-Moliner V, Moranta D, Dahlström KM, Regueiro V, Tomás A, Cano V, Pérez-Gutiérrez C, Frank CG, Fernández-Carrasco H, Insua JL, Salminen TA, Garmendia J, Bengoechea JA.** 2 November 2015. Deciphering tissue-induced Klebsiella pneumoniae lipid A structure. Proc Natl Acad Sci U S A http://dx.doi.org/10.1073/pnas.1508820112.
264. **Jiang GZ, Sugiyama T, Kato Y, Koide N, Yokochi T.** 1995. Binding of mannose-binding protein to Klebsiella O3 lipopolysaccharide possessing the mannose homopolysaccharide as the O-specific polysaccharide and its relation to complement activation. Infect Immun **63:**2537–2540.
265. **Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW.** 2000. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun **68:**688–693. http://dx.doi.org/10.1128/IAI.68.2.688-693.2000.
266. **Shankar-Sinha S, Valencia GA, Janes BK, Rosenberg JK, Whitfield C, Bender RA, Standiford TJ, Younger JG.** 2004. The Klebsiella pneumoniae O antigen contributes to bacteremia and lethality during murine pneumonia. Infect Immun **72:**1423–1430. http://dx.doi.org/10.1128/IAI.72.3.1423-1430.2004.
267. **Regue M, Hita B, Pique N, Izquierdo L, Merino S, Fresno S, Benedi VJ, Tomas JM.** 2004. A gene, uge, is essential for Klebsiella pneumoniae virulence. Infect Immun **72:**54–61. http://dx.doi.org/10.1128/IAI.72.1.54-61.2004.
268. **Izquierdo L, Coderch N, Pique N, Bedini E, Corsaro MM, Merino S, Fresno S, Tomas JM, Regue M.** 2003. The Klebsiella pneumoniae wabG gene: role in biosynthesis of the core lipopolysaccharide and virulence. J Bacteriol **185:**7213–7221. http://dx.doi.org/10.1128/JB.185.24.7213-7221.2003.
269. **Andrade LN, Vitali L, Gaspar GG, Bellissimo-Rodrigues F, Martinez R, Darini AL.** 2014. Expansion and evolution of a virulent, extensively drug-resistant (polymyxin B-resistant), QnrS1-, CTX-M-2-, and KPC-2-producing Klebsiella pneumoniae ST11 international high-risk clone. J Clin Microbiol **52:**2530–2535. http://dx.doi.org/10.1128/JCM.00088-14.
270. **Lin WH, Kao CY, Yang DC, Tseng CC, Wu AB, Teng CH, Wang MC, Wu JJ.** 2014. Clinical and microbiological characteristics of Klebsiella pneumoniae from community-acquired recurrent urinary tract infections. Eur J Clin Microbiol Infect Dis **33:**1533–1539. http://dx.doi.org/10.1007/s10096-014-2100-4.
271. **Fresno S, Jimenez N, Canals R, Merino S, Corsaro MM, Lanzetta R, Parrilli M, Pieretti G, Regue M, Tomas JM.** 2007. A second galacturonic acid transferase is required for core lipopolysaccharide biosynthesis and complete capsule association with the cell surface in Klebsiella pneumoniae. J Bacteriol **189:**1128–1137. http://dx.doi.org/10.1128/JB.01489-06.
272. **Candan ED, Aksoz N.** 2015. Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors. Acta Biochim Pol **62:**867–874. http://dx.doi.org/10.18388/abp.2015_1148.
273. **Huynh DT, Kim AY, Seol IH, Jung S, Lim MC, Lee JA, Jo MR, Choi SJ, Kim B, Lee J, Kim W, Kim YR.** 2015. Inactivation of the virulence factors from 2,3-butanediol-producing Klebsiella pneumoniae. Appl Microbiol Biotechnol **99:**9427–9438. http://dx.doi.org/10.1007/s00253-015-6861-1.
274. **Jung SW, Chae HJ, Park YJ, Yu JK, Kim SY, Lee HK, Lee JH, Kahng JM, Lee SO, Lee MK, Lim JH, Lee CH, Chang SJ, Ahn JY, Lee JW, Park YG.** 2013. Microbiological and clinical characteristics of bacteraemia caused by the hypermucoviscosity phenotype of Klebsiella pneumoniae in Korea. Epidemiol Infect **141:**334–340. http://dx.doi.org/10.1017/S0950268812000933.
275. **Jung SG, Jang JH, Kim AY, Lim MC, Kim B, Lee J, Kim YR.** 2013. Removal of pathogenic factors from 2,3-butanediol-producing Klebsiella species by inactivating virulence-related wabG gene. Appl Microbiol Biotechnol **97:**1997–2007. http://dx.doi.org/10.1007/s00253-012-4284-9.
276. **Di Martino P, Livrelli V, Sirot D, Joly B, Darfeuille-Michaud A.** 1996. A new fimbrial antigen harbored by CAZ-5/SHV-4-producing Klebsiella pneumoniae strains involved in nosocomial infections. Infect Immun **64:**2266–2273.
277. **Darfeuille-Michaud A, Jallat C, Aubel D, Sirot D, Rich C, Sirot J, Joly B.** 1992. R-plasmid-encoded adhesive factor in Klebsiella pneumoniae strains responsible for human nosocomial infections. Infect Immun **60:**44–55.
278. **Favre-Bonte S, Darfeuille-Michaud A, Forestier C.** 1995. Aggregative adherence of Klebsiella pneumoniae to human intestine-407 cells. Infect Immun **63:**1318–1328.
279. **Stahlhut SG, Tchesnokova V, Struve C, Weissman SJ, Chattopadhyay S, Yakovenko O, Aprikian P, Sokurenko EV, Krogfelt KA.** 2009. Comparative structure-function analysis of mannose-specific FimH adhesins from Klebsiella pneumoniae and Escherichia coli. J Bacteriol **191:**6592–6601. http://dx.doi.org/10.1128/JB.00786-09.
280. **Klemm P, Schembri MA.** 2000. Fimbrial surface display systems in bacteria: from vaccines to random libraries. Microbiology **146**(Part 12):3025–3032. http://dx.doi.org/10.1099/00221287-146-12-3025.
281. **Gerlach GF, Clegg S, Allen BL.** 1989. Identification and characterization of the genes encoding the type 3 and type 1 fimbrial adhesins of Klebsiella pneumoniae. J Bacteriol **171:**1262–1270.
282. **Jones CH, Pinkner JS, Roth R, Heuser J, Nicholes AV, Abraham SN, Hultgren SJ.** 1995. FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the Enterobacteriaceae. Proc Natl Acad Sci U S A **92:**2081–2085. http://dx.doi.org/10.1073/pnas.92.6.2081.
283. **Rosen DA, Hilliard JK, Tiemann KM, Todd EM, Morley SC, Hunstad DA.** 7 September 2015. Klebsiella pneumoniae FimK promotes virulence in murine pneumonia. J Infect Dis http://dx.doi.org/10.1093/infdis/jiv440.
284. **Kline KA, Falker S, Dahlberg S, Normark S, Henriques-Normark B.** 2009. Bacterial adhesins in host-microbe interactions. Cell Host Microbe **5:**580–592. http://dx.doi.org/10.1016/j.chom.2009.05.011.
285. **Firon N, Ofek I, Sharon N.** 1984. Carbohydrate-binding sites of the mannose-specific fimbrial lectins of enterobacteria. Infect Immun **43:**1088–1090.
286. **Tarkkanen AM, Westerlund-Wikstrom B, Erkkila L, Korhonen TK.** 1998. Immunohistological localization of the MrkD adhesin in the type 3 fimbriae of Klebsiella pneumoniae. Infect Immun **66:**2356–2361.
287. **Allen BL, Gerlach GF, Clegg S.** 1991. Nucleotide sequence and functions of mrk determinants necessary for expression of type 3 fimbriae in Klebsiella pneumoniae. J Bacteriol **173:**916–920.
288. **Sebghati TA, Korhonen TK, Hornick DB, Clegg S.** 1998. Characterization of the type 3 fimbrial adhesins of Klebsiella strains. Infect Immun **66:**2887–2894.
289. **Struve C, Bojer M, Krogfelt KA.** 2009. Identification of a conserved chromosomal region encoding Klebsiella pneumoniae type 1 and type 3 fimbriae and assessment of the role of fimbriae in pathogenicity. Infect Immun **77:**5016–5024. http://dx.doi.org/10.1128/IAI.00585-09.

290. **Tarkkanen AM, Virkola R, Clegg S, Korhonen TK.** 1997. Binding of the type 3 fimbriae of Klebsiella pneumoniae to human endothelial and urinary bladder cells. Infect Immun **65:**1546–1549.
291. **Wurker M, Beuth J, Ko HL, Przondo-Mordarska A, Pulverer G.** 1990. Type of fimbriation determines adherence of Klebsiella bacteria to human epithelial cells. Zentralbl Bakteriol **274:**239–245. http://dx.doi.org/10.1016/S0934-8840(11)80106-4.
292. **Hornick DB, Allen BL, Horn MA, Clegg S.** 1992. Adherence to respiratory epithelia by recombinant Escherichia coli expressing Klebsiella pneumoniae type 3 fimbrial gene products. Infect Immun **60:**1577–1588.
293. **Davies D.** 2003. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov **2:**114–122. http://dx.doi.org/10.1038/nrd1008.
294. **Stahlhut SG, Struve C, Krogfelt KA, Reisner A.** 2012. Biofilm formation of Klebsiella pneumoniae on urethral catheters requires either type 1 or type 3 fimbriae. FEMS Immunol Med Microbiol **65:**350–359. http://dx.doi.org/10.1111/j.1574-695X.2012.00965.x.
295. **Schroll C, Barken KB, Krogfelt KA, Struve C.** 2010. Role of type 1 and type 3 fimbriae in Klebsiella pneumoniae biofilm formation. BMC Microbiol **10:**179. http://dx.doi.org/10.1186/1471-2180-10-179.
296. **Jagnow J, Clegg S.** 2003. Klebsiella pneumoniae MrkD-mediated biofilm formation on extracellular matrix- and collagen-coated surfaces. Microbiology **149:**2397–2405. http://dx.doi.org/10.1099/mic.0.26434-0.
297. **Schembri MA, Blom J, Krogfelt KA, Klemm P.** 2005. Capsule and fimbria interaction in Klebsiella pneumoniae. Infect Immun **73:**4626–4633. http://dx.doi.org/10.1128/IAI.73.8.4626-4633.2005.
298. **Schembri MA, Klemm P.** 2001. Biofilm formation in a hydrodynamic environment by novel fimH variants and ramifications for virulence. Infect Immun **69:**1322–1328. http://dx.doi.org/10.1128/IAI.69.3.1322-1328.2001.
299. **Ramirez P, Bassi GL, Torres A.** 2012. Measures to prevent nosocomial infections during mechanical ventilation. Curr Opin Crit Care **18:**86–92. http://dx.doi.org/10.1097/MCC.0b013e32834ef3ff.
300. **Kollef MH.** 2004. Prevention of hospital-associated pneumonia and ventilator-associated pneumonia. Crit Care Med **32:**1396–1405. http://dx.doi.org/10.1097/01.CCM.0000128569.09113.FB.
301. **Francois P, Vaudaux P, Lew PD.** 1998. Role of plasma and extracellular matrix proteins in the physiopathology of foreign body infections. Ann Vasc Surg **12:**34–40. http://dx.doi.org/10.1007/s100169900112.
302. **Murphy CN, Mortensen MS, Krogfelt KA, Clegg S.** 2013. Role of Klebsiella pneumoniae type 1 and type 3 fimbriae in colonizing silicone tubes implanted into the bladders of mice as a model of catheter-associated urinary tract infections. Infect Immun **81:**3009–3017. http://dx.doi.org/10.1128/IAI.00348-13.
303. **Gbarah A, Gahmberg CG, Ofek I, Jacobi U, Sharon N.** 1991. Identification of the leukocyte adhesion molecules CD11 and CD18 as receptors for type 1-fimbriated (mannose-specific) Escherichia coli. Infect Immun **59:**4524–4530.
304. **Athamna A, Ofek I.** 1988. Enzyme-linked immunosorbent assay for quantitation of attachment and ingestion stages of bacterial phagocytosis. J Clin Microbiol **26:**62–66.
305. **Malaviya R, Ikeda T, Ross E, Abraham SN.** 1996. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. Nature **381:**77–80. http://dx.doi.org/10.1038/381077a0.
306. **Przondo-Mordarska A, Ko HL, Beuth J, Gamian A, Pulverer G.** 1991. Chemiluminescence response of human polymorphonuclear leukocytes induced by purified, latex attached Klebsiella fimbriae. Zentralbl Bakteriol **275:**521–529. http://dx.doi.org/10.1016/S0934-8840(11)80174-X.
307. **Sahly H, Podschun R, Oelschlaeger TA, Greiwe M, Parolis H, Hasty D, Kekow J, Ullmann U, Ofek I, Sela S.** 2000. Capsule impedes adhesion to and invasion of epithelial cells by Klebsiella pneumoniae. Infect Immun **68:**6744–6749. http://dx.doi.org/10.1128/IAI.68.12.6744-6749.2000.
308. **Favre-Bonte S, Joly B, Forestier C.** 1999. Consequences of reduction of Klebsiella pneumoniae capsule expression on interactions of this bacterium with epithelial cells. Infect Immun **67:**554–561.
309. **Bullen JJ, Rogers HJ, Griffiths E.** 1972. Iron binding proteins and infection. Br J Haematol **23:**389–392. http://dx.doi.org/10.1111/j.1365-2141.1972.tb07073.x.
310. **Carniel E.** 2001. The Yersinia high-pathogenicity island: an iron-uptake island. Microbes Infect **3:**561–569. http://dx.doi.org/10.1016/S1286-4579(01)01412-5.
311. **Miethke M, Marahiel MA.** 2007. Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev **71:**413–451. http://dx.doi.org/10.1128/MMBR.00012-07.
312. **Bachman MA, Lenio S, Schmidt L, Oyler JE, Weiser JN.** 2012. Interaction of lipocalin 2, transferrin, and siderophores determines the replicative niche of Klebsiella pneumoniae during pneumonia. mBio **3:**e00224–11. http://dx.doi.org/10.1128/mBio.00224-11.
313. **Brock JH, Williams PH, Liceaga J, Wooldridge KG.** 1991. Relative availability of transferrin-bound iron and cell-derived iron to aerobactin-producing and enterochelin-producing strains of Escherichia coli and to other microorganisms. Infect Immun **59:**3185–3190.
314. **Perry RD, Balbo PB, Jones HA, Fetherston JD, DeMoll E.** 1999. Yersiniabactin from Yersinia pestis: biochemical characterization of the siderophore and its role in iron transport and regulation. Microbiology **145**(Part 5)**:**1181–1190.
315. **Podschun R, Sievers D, Fischer A, Ullmann U.** 1993. Serotypes, hemagglutinins, siderophore synthesis, and serum resistance of Klebsiella isolates causing human urinary tract infections. J Infect Dis **168:**1415–1421. http://dx.doi.org/10.1093/infdis/168.6.1415.
316. **Tarkkanen AM, Allen BL, Williams PH, Kauppi M, Haahtela K, Siitonen A, Orskov I, Orskov F, Clegg S, Korhonen TK.** 1992. Fimbriation, capsulation, and iron-scavenging systems of Klebsiella strains associated with human urinary tract infection. Infect Immun **60:**1187–1192.
317. **Koczura R, Kaznowski A.** 2003. Occurrence of the Yersinia high-pathogenicity island and iron uptake systems in clinical isolates of Klebsiella pneumoniae. Microb Pathog **35:**197–202. http://dx.doi.org/10.1016/S0882-4010(03)00125-6.
318. **El Fertas-Aissani R, Messai Y, Alouache S, Bakour R.** 2013. Virulence profiles and antibiotic susceptibility patterns of Klebsiella pneumoniae strains isolated from different clinical specimens. Pathol Biol (Paris) **61:**209–216. http://dx.doi.org/10.1016/j.patbio.2012.10.004.
319. **Hsieh PF, Lin TL, Lee CZ, Tsai SF, Wang JT.** 2008. Serum-induced iron-acquisition systems and TonB contribute to virulence in Klebsiella pneumoniae causing primary pyogenic liver abscess. J Infect Dis **197:**1717–1727. http://dx.doi.org/10.1086/588383.
320. **Müller S, Valdebenito M, Hantke K.** 2009. Salmochelin, the long-overlooked catecholate siderophore of Salmonella. Biometals **22:**691–695. http://dx.doi.org/10.1007/s10534-009-9217-4.
321. **Lawlor MS, O'Connor C, Miller VL.** 2007. Yersiniabactin is a virulence factor for Klebsiella pneumoniae during pulmonary infection. Infect Immun **75:**1463–1472. http://dx.doi.org/10.1128/IAI.00372-06.
322. **Lai YC, Peng HL, Chang HY.** 2001. Identification of genes induced in vivo during Klebsiella pneumoniae CG43 infection. Infect Immun **69:**7140–7145. http://dx.doi.org/10.1128/IAI.69.11.7140-7145.2001.
323. **Cowland JB, Borregaard N.** 1997. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics **45:**17–23. http://dx.doi.org/10.1006/geno.1997.4896.
324. **Chan YR, Liu JS, Pociask DA, Zheng M, Mietzner TA, Berger T, Mak TW, Clifton MC, Strong RK, Ray P, Kolls JK.** 2009. Lipocalin 2 is required for pulmonary host defense against Klebsiella infection. J Immunol **182:**4947–4956. http://dx.doi.org/10.4049/jimmunol.0803282.
325. **Nelson AL, Barasch JM, Bunte RM, Weiser JN.** 2005. Bacterial colonization of nasal mucosa induces expression of siderocalin, an iron-sequestering component of innate immunity. Cell Microbiol **7:**1404–1417. http://dx.doi.org/10.1111/j.1462-5822.2005.00566.x.
326. **Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK.** 2002. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell **10:**1033–1043. http://dx.doi.org/10.1016/S1097-2765(02)00708-6.
327. **Bachman MA, Miller VL, Weiser JN.** 2009. Mucosal lipocalin 2 has pro-inflammatory and iron-sequestering effects in response to bacterial enterobactin. PLoS Pathog **5:**e1000622. http://dx.doi.org/10.1371/journal.ppat.1000622.
328. **Wasserman SI, Soter NA, Center DM, Austen KF.** 1977. Cold urticaria. Recognition and characterization of a neutrophil chemotactic factor which appears in serum during experimental cold challenge. J Clin Invest **60:**189–196.
329. **Bachman MA, Oyler JE, Burns SH, Caza M, Lepine F, Dozois CM, Weiser JN.** 2011. Klebsiella pneumoniae yersiniabactin promotes respiratory tract infection through evasion of lipocalin 2. Infect Immun **79:**3309–3316. http://dx.doi.org/10.1128/IAI.05114-11.
330. **Bach S, de Almeida A, Carniel E.** 2000. The Yersinia high-pathogenicity

island is present in different members of the family Enterobacteriaceae. FEMS Microbiol Lett **183:**289–294. http://dx.doi.org/10.1111/j.1574-6968.2000.tb08973.x.

331. **Russo TA, Olson R, MacDonald U, Beanan J, Davidson BA.** 8 June 2015. Aerobactin, but not yersiniabactin, salmochelin, or enterobactin, enables the growth/survival of hypervirulent (hypermucoviscous) Klebsiella pneumoniae ex vivo and in vivo. Infect Immun http://dx.doi.org/10.1128/IAI.00430-15.
332. **Fischbach MA, Lin H, Zhou L, Yu Y, Abergel RJ, Liu DR, Raymond KN, Wanner BL, Strong RK, Walsh CT, Aderem A, Smith KD.** 2006. The pathogen-associated iroA gene cluster mediates bacterial evasion of lipocalin 2. Proc Natl Acad Sci U S A **103:**16502–16507. http://dx.doi.org/10.1073/pnas.0604636103.
333. **Fischbach MA, Lin H, Liu DR, Walsh CT.** 2005. In vitro characterization of IroB, a pathogen-associated C-glycosyltransferase. Proc Natl Acad Sci U S A **102:**571–576. http://dx.doi.org/10.1073/pnas.0408463102.
334. **Hantke K, Nicholson G, Rabsch W, Winkelmann G.** 2003. Salmochelins, siderophores of Salmonella enterica and uropathogenic Escherichia coli strains, are recognized by the outer membrane receptor IroN. Proc Natl Acad Sci U S A **100:**3677–3682. http://dx.doi.org/10.1073/pnas.0737682100.
335. **Vernet V, Philippon A, Madoulet C, Vistelle R, Jaussaud R, Chippaux C.** 1995. Virulence factors (aerobactin and mucoid phenotype) in Klebsiella pneumoniae and Escherichia coli blood culture isolates. FEMS Microbiol Lett **130:**51–57. http://dx.doi.org/10.1111/j.1574-6968.1995.tb07697.x.
336. **Nassif X, Sansonetti PJ.** 1986. Correlation of the virulence of Klebsiella pneumoniae K1 and K2 with the presence of a plasmid encoding aerobactin. Infect Immun **54:**603–608.
337. **Chen YT, Chang HY, Lai YC, Pan CC, Tsai SF, Peng HL.** 2004. Sequencing and analysis of the large virulence plasmid pLVPK of Klebsiella pneumoniae CG43. Gene **337:**189–198. http://dx.doi.org/10.1016/j.gene.2004.05.008.
338. **Tang HL, Chiang MK, Liou WJ, Chen YT, Peng HL, Chiou CS, Liu KS, Lu MC, Tung KC, Lai YC.** 2010. Correlation between Klebsiella pneumoniae carrying pLVPK-derived loci and abscess formation. Eur J Clin Microbiol Infect Dis **29:**689–698. http://dx.doi.org/10.1007/s10096-010-0915-1.
339. **Hsieh PF, Liu JY, Pan YJ, Wu MC, Lin TL, Huang YT, Wang JT.** 2013. Klebsiella pneumoniae peptidoglycan-associated lipoprotein and murein lipoprotein contribute to serum resistance, antiphagocytosis, and proinflammatory cytokine stimulation. J Infect Dis **208:**1580–1589. http://dx.doi.org/10.1093/infdis/jit384.
340. **Pichavant M, Delneste Y, Jeannin P, Fourneau C, Brichet A, Tonnel AB, Gosset P.** 2003. Outer membrane protein A from Klebsiella pneumoniae activates bronchial epithelial cells: implication in neutrophil recruitment. J Immunol **171:**6697–6705. http://dx.doi.org/10.4049/jimmunol.171.12.6697.
341. **Jeannin P, Bottazzi B, Sironi M, Doni A, Rusnati M, Presta M, Maina V, Magistrelli G, Haeuw JF, Hoeffel G, Thieblemont N, Corvaia N, Garlanda C, Delneste Y, Mantovani A.** 2005. Complexity and complementarity of outer membrane protein A recognition by cellular and humoral innate immunity receptors. Immunity **22:**551–560. http://dx.doi.org/10.1016/j.immuni.2005.03.008.
342. **Jeannin P, Magistrelli G, Goetsch L, Haeuw JF, Thieblemont N, Bonnefoy JY, Delneste Y.** 2002. Outer membrane protein A (OmpA): a new pathogen-associated molecular pattern that interacts with antigen presenting cells—impact on vaccine strategies. Vaccine **20**(Suppl 4)**:** A23–A27. http://dx.doi.org/10.1016/S0264-410X(02)00383-3.
343. **Llobet E, March C, Gimenez P, Bengoechea JA.** 2009. Klebsiella pneumoniae OmpA confers resistance to antimicrobial peptides. Antimicrob Agents Chemother **53:**298–302. http://dx.doi.org/10.1128/AAC.00657-08.
344. **March C, Moranta D, Regueiro V, Llobet E, Tomas A, Garmendia J, Bengoechea JA.** 2011. Klebsiella pneumoniae outer membrane protein A is required to prevent the activation of airway epithelial cells. J Biol Chem **286:**9956–9967. http://dx.doi.org/10.1074/jbc.M110.181008.
345. **Chen JH, Siu LK, Fung CP, Lin JC, Yeh KM, Chen TL, Tsai YK, Chang FY.** 2010. Contribution of outer membrane protein K36 to antimicrobial resistance and virulence in Klebsiella pneumoniae. J Antimicrob Chemother **65:**986–990. http://dx.doi.org/10.1093/jac/dkq056.
346. **Shin SY, Bae IK, Kim J, Jeong SH, Yong D, Kim JM, Lee K.** 2012. Resistance to carbapenems in sequence type 11 Klebsiella pneumoniae is related to DHA-1 and loss of OmpK35 and/or OmpK36. J Med Microbiol **61:**239–245. http://dx.doi.org/10.1099/jmm.0.037036-0.
347. **Hernandez-Alles S, Alberti S, Alvarez D, Domenech-Sanchez A, Martinez-Martinez L, Gil J, Tomas JM, Benedi VJ.** 1999. Porin expression in clinical isolates of Klebsiella pneumoniae. Microbiology **145**(Part 3)**:** 673–679.
348. **Garcia-Fernandez A, Miriagou V, Papagiannitsis CC, Giordano A, Venditti M, Mancini C, Carattoli A.** 2010. An ertapenem-resistant extended-spectrum-beta-lactamase-producing Klebsiella pneumoniae clone carries a novel OmpK36 porin variant. Antimicrob Agents Chemother **54:**4178–4184. http://dx.doi.org/10.1128/AAC.01301-09.
349. **Chudackova E, Bergerova T, Fajfrlik K, Cervena D, Urbaskova P, Empel J, Gniadkowski M, Hrabak J.** 2010. Carbapenem-nonsusceptible strains of Klebsiella pneumoniae producing SHV-5 and/or DHA-1 beta-lactamases in a Czech hospital. FEMS Microbiol Lett **309:**62–70. http://dx.doi.org/10.1111/j.1574-6968.2010.02016.x.
350. **Mena A, Plasencia V, Garcia L, Hidalgo O, Ayestaran JI, Alberti S, Borrell N, Perez JL, Oliver A.** 2006. Characterization of a large outbreak by CTX-M-1-producing Klebsiella pneumoniae and mechanisms leading to in vivo carbapenem resistance development. J Clin Microbiol **44:** 2831–2837. http://dx.doi.org/10.1128/JCM.00418-06.
351. **Doumith M, Ellington MJ, Livermore DM, Woodford N.** 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and Enterobacter spp. clinical isolates from the UK. J Antimicrob Chemother **63:**659–667. http://dx.doi.org/10.1093/jac/dkp029.
352. **Hernandez-Alles S, Conejo M, Pascual A, Tomas JM, Benedi VJ, Martinez-Martinez L.** 2000. Relationship between outer membrane alterations and susceptibility to antimicrobial agents in isogenic strains of Klebsiella pneumoniae. J Antimicrob Chemother **46:**273–277. http://dx.doi.org/10.1093/jac/46.2.273.
353. **Tsai YK, Fung CP, Lin JC, Chen JH, Chang FY, Chen TL, Siu LK.** 2011. Klebsiella pneumoniae outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. Antimicrob Agents Chemother **55:**1485–1493. http://dx.doi.org/10.1128/AAC.01275-10.
354. **March C, Cano V, Moranta D, Llobet E, Perez-Gutierrez C, Tomas JM, Suarez T, Garmendia J, Bengoechea JA.** 2013. Role of bacterial surface structures on the interaction of Klebsiella pneumoniae with phagocytes. PLoS One **8:**e56847. http://dx.doi.org/10.1371/journal.pone.0056847.
355. **Padilla E, Llobet E, Domenech-Sanchez A, Martinez-Martinez L, Bengoechea JA, Alberti S.** 2010. Klebsiella pneumoniae AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrob Agents Chemother **54:**177–183. http://dx.doi.org/10.1128/AAC.00715-09.
356. **Wang X, Chen H, Zhang Y, Wang Q, Zhao C, Li H, He W, Zhang F, Wang Z, Li S, Wang H.** 2015. Genetic characterisation of clinical Klebsiella pneumoniae isolates with reduced susceptibility to tigecycline: role of the global regulator RamA and its local repressor RamR. Int J Antimicrob Agents **45:**635–640. http://dx.doi.org/10.1016/j.ijantimicag.2014.12.022.
357. **Bialek-Davenet S, Lavigne JP, Guyot K, Mayer N, Tournebize R, Brisse S, Leflon-Guibout V, Nicolas-Chanoine MH.** 2015. Differential contribution of AcrAB and OqxAB efflux pumps to multidrug resistance and virulence in Klebsiella pneumoniae. J Antimicrob Chemother **70:**81–88. http://dx.doi.org/10.1093/jac/dku340.
358. **Zhong X, Xu H, Chen D, Zhou H, Hu X, Cheng G.** 2014. First emergence of acrAB and oqxAB mediated tigecycline resistance in clinical isolates of Klebsiella pneumoniae pre-dating the use of tigecycline in a Chinese hospital. PLoS One **9:**e115185. http://dx.doi.org/10.1371/journal.pone.0115185.
359. **Ma LC, Fang CT, Lee CZ, Shun CT, Wang JT.** 2005. Genomic heterogeneity in Klebsiella pneumoniae strains is associated with primary pyogenic liver abscess and metastatic infection. J Infect Dis **192:**117–128. http://dx.doi.org/10.1086/430619.
360. **Vogels GD, Van der Drift C.** 1976. Degradation of purines and pyrimidines by microorganisms. Bacteriol Rev **40:**403–468.
361. **Chou HC, Lee CZ, Ma LC, Fang CT, Chang SC, Wang JT.** 2004. Isolation of a chromosomal region of Klebsiella pneumoniae associated with allantoin metabolism and liver infection. Infect Immun **72:**3783–3792. http://dx.doi.org/10.1128/IAI.72.7.3783-3792.2004.
362. **Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailloud F, Kassis-**

**Chikhani N, Arlet G, Decre D.** 2014. Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of Klebsiella pneumoniae. J Clin Microbiol **52:**4377–4380. http://dx.doi.org/10.1128/JCM.02316-14.

363. **Bachman MA, Breen P, Deornellas V, Mu Q, Zhao L, Wu W, Cavalcoli JD, Mobley HLT.** 2015. Genome-wide identification of Klebsiella pneumoniae fitness genes during lung infection. mBio **6:**e00775-15. http://dx.doi.org/10.1128/mBio.00775-15.
364. **Maroncle N, Balestrino D, Rich C, Forestier C.** 2002. Identification of Klebsiella pneumoniae genes involved in intestinal colonization and adhesion using signature-tagged mutagenesis. Infect Immun **70:**4729–4734. http://dx.doi.org/10.1128/IAI.70.8.4729-4734.2002.
365. **Struve C, Forestier C, Krogfelt KA.** 2003. Application of a novel multi-screening signature-tagged mutagenesis assay for identification of Klebsiella pneumoniae genes essential in colonization and infection. Microbiology **149:**167–176. http://dx.doi.org/10.1099/mic.0.25833-0.
366. **Tu YC, Lu MC, Chiang MK, Huang SP, Peng HL, Chang HY, Jan MS, Lai YC.** 2009. Genetic requirements for Klebsiella pneumoniae-induced liver abscess in an oral infection model. Infect Immun **77:**2657–2671. http://dx.doi.org/10.1128/IAI.01523-08.
367. **Lery LM, Frangeul L, Tomas A, Passet V, Almeida AS, Bialek-Davenet S, Barbe V, Bengoechea JA, Sansonetti P, Brisse S, Tournebize R.** 2014. Comparative analysis of Klebsiella pneumoniae genomes identifies a phospholipase D family protein as a novel virulence factor. BMC Biol **12:**41. http://dx.doi.org/10.1186/1741-7007-12-41.
368. **Tomas A, Lery L, Regueiro V, Perez-Gutierrez C, Martinez V, Moranta D, Llobet E, Gonzalez-Nicolau M, Insua JL, Tomas JM, Sansonetti PJ, Tournebize R, Bengoechea JA.** 13 May 2015. Functional genomic screen identifies Klebsiella pneumoniae factors implicated in blocking NF-kappaB signalling. J Biol Chem http://dx.doi.org/10.1074/jbc.M114.621292.
369. **Lavender HF, Jagnow JR, Clegg S.** 2004. Biofilm formation in vitro and virulence in vivo of mutants of Klebsiella pneumoniae. Infect Immun **72:**4888–4890. http://dx.doi.org/10.1128/IAI.72.8.4888-4890.2004.
370. **Boddicker JD, Anderson RA, Jagnow J, Clegg S.** 2006. Signature-tagged mutagenesis of Klebsiella pneumoniae to identify genes that influence biofilm formation on extracellular matrix material. Infect Immun **74:**4590–4597. http://dx.doi.org/10.1128/IAI.00129-06.
371. **Wu MC, Lin TL, Hsieh PF, Yang HC, Wang JT.** 2011. Isolation of genes involved in biofilm formation of a Klebsiella pneumoniae strain causing pyogenic liver abscess. PLoS One **6:**e23500. http://dx.doi.org/10.1371/journal.pone.0023500.
372. **Nassif X, Fournier JM, Arondel J, Sansonetti PJ.** 1989. Mucoid phenotype of Klebsiella pneumoniae is a plasmid-encoded virulence factor. Infect Immun **57:**546–552.
373. **Frank CG, Reguerio V, Rother M, Moranta D, Maeurer AP, Garmendia J, Meyer TF, Bengoechea JA.** 2013. Klebsiella pneumoniae targets an EGF receptor-dependent pathway to subvert inflammation. Cell Microbiol **15:**1212–1233. http://dx.doi.org/10.1111/cmi.12110.
374. **Maroncle N, Rich C, Forestier C.** 2006. The role of Klebsiella pneumoniae urease in intestinal colonization and resistance to gastrointestinal stress. Res Microbiol **157:**184–193. http://dx.doi.org/10.1016/j.resmic.2005.06.006.
375. **Yu VL, Hansen DS, Ko WC, Sagnimeni A, Klugman KP, von Gottberg A, Goossens H, Wagener MM, Benedi VJ.** 2007. Virulence characteristics of Klebsiella and clinical manifestations of K. pneumoniae bloodstream infections. Emerg Infect Dis **13:**986–993. http://dx.doi.org/10.3201/eid1307.070187.