**Klebsiella pneumoniae** is a ubiquitous Gram-negative encapsulated bacterium that resides in the mucosal surfaces of mammals and the environment (soil, water, etc.). In humans, *K. pneumoniae* colonizes the gastrointestinal tract and less frequently the nasopharynx, whence it gains entry to the circulation and other tissues causing infection.

In the pre-antibiotic era, *K. pneumoniae* was an important cause of community-acquired pneumonia, especially in alcoholics and diabetics. In the antibiotic era, *K. pneumoniae* became established in hospitals as a leading cause of healthcare-associated infections.1

In pediatric wards, it causes sepsis and meningitis in premature neonates and infants as well as serious infections in immunocompromised and malnourished children, whereas in the community, *K. pneumoniae* is a common cause of urinary tract infections among immunocompetent children.

In recent years, most *K. pneumoniae* infections are caused by strains termed “classic” *K. pneumoniae* (cKp). These strains persist in hospital environments and cause infections in debilitated patients. cKp strains appear to be distinct from hypervirulent *K. pneumoniae* (hvKp), a variant that was first described in the Asian Pacific Rim to cause community-acquired, invasive and metastatic infections, including liver abscesses, endophthalmitis, meningesis and septic arthritis in diabetics and immunocompetent young individuals.2-3 The emergence and spread of new multidrug-resistant (MDR) clones and the international dissemination of hvKp strains have renewed interest in *K. pneumoniae*.

**VIRULENCE**

To establish infection, *K. pneumoniae* must overcome mechanical and chemical barriers and escape host humoral and cellular innate defenses. After gaining access to the host, the invading organisms are recognized by the immune cells through the pattern recognition receptors and trigger production of various immune mediators. Central role in the innate immune response plays the monocyte/macrophage system, which has phagocytic capabilities and orchestrates the immune response through cytokine and chemokine production (Fig. 1). Among the effector cells that are recruited first to the infection site are the neutrophils. Important mediators involved in this process are interleukin (IL)-8 and IL-23, which induces production of IL-17 that promotes granulopoietic response.6-7 IL-12 also amplifies the expression of IL-17 through production of interferon-gamma. Other factors participating in the immune response are the production of IL-1β via activation of the NOD-like receptor pyrin domain containing (NLRP3) inflammasome pathway and the production of other pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-α) and IL-6.8

The specific factors that *K. pneumoniae* employs to circumvent the immune defenses are not fully elucidated. Presently, there are 4 well-characterized virulence factors, namely the fimbriae, the capsule, the lipopolysaccharide (LPS) and siderophores.6,9 *K. pneumoniae* is equipped with adhesins, type 1 and type 3 fimbriae, which facilitate adherence to epithelial and immune cells as well as to abiotic surfaces (Fig. 1). Based on the composition of capsular polysaccharides (CPs) of *K. pneumoniae*, 78 distinct capsular serotypes (K1 to K78) have been recognized. Of note, the vast majority of hvKp strains belong to serotypes K1 and K2. The high level of virulence of hvKp strains is, at least partially, due to excess production of capsular material (hypermucoviscous phenotype). Although the capsule composition plays an important role in protecting *K. pneumoniae* against the host immune response, it is likely that other factors contribute

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**Key Words**: *Klebsiella pneumoniae*, virulence, biofilm, resistance

Klebsiella pneumoniae: Virulence, Biofilm and Antimicrobial Resistance

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to virulence as well. Indeed, certain *K. pneumoniae* strains may modify the LPS to a degree that is not recognized by the host cells and others may use capsule to mask LPS from detection by toll-like receptor (TLR4) receptors. *K. pneumoniae* is equipped with types 1 and 3 fimbriae mediating adhesion to biotic and abiotic surfaces facilitating epithelial cell invasion and biofilm formation. It also synthesizes siderophores (enterobactin, aerobactin, yersiniabactin and salmochelin) to acquire iron from the host. The monocyte/macrophage system plays a central role in the innate immune response, through phagocytosis and production of immune mediators such as cytokines and chemokines. Important mediator in this process is IL-23, that induces IL-17 production which along with IL-8 promote neutrophil recruitment. IL-17 expression is also amplified by IL-12 through IFN-γ. Other important cytokines are IL-1β, produced via activation of the NOD-like receptor pyrin domain-containing (NLRP3) inflammasome pathway, and other pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6. Perpendicular line ( ────│) indicates inhibition. Adapted from Paczosa and Mecsas.6 IFN-γ, interferon-gamma.

**FIGURE 1.** Schematic presentation of *K. pneumoniae* virulence factors and of host innate immune response. Capsular polysaccharides prevent phagocytosis and block complement-mediated lysis and opsonization. The intact LPS elicits a robust inflammatory response and prevents binding of C1q to bacteria and the subsequent activation of the complement pathway. Certain strains may modify the LPS making it unrecognizable to immune cells, whereas others may use the capsule to prevent LPS detection by toll-like receptor (TLR4) receptors. *K. pneumoniae* is equipped with types 1 and 3 fimbriae mediating adhesion to biotic and abiotic surfaces facilitating epithelial cell invasion and biofilm formation. It also synthesizes siderophores (enterobactin, aerobactin, yersiniabactin and salmochelin) to acquire iron from the host. The monocyte/macrophage system plays a central role in the innate immune response, through phagocytosis and production of immune mediators such as cytokines and chemokines. Important mediator in this process is IL-23, that induces IL-17 production which along with IL-8 promote neutrophil recruitment. IL-17 expression is also amplified by IL-12 through IFN-γ. Other important cytokines are IL-1β, produced via activation of the NOD-like receptor pyrin domain-containing (NLRP3) inflammasome pathway, and other pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6. Perpendicular line ( ────│) indicates inhibition. Adapted from Paczosa and Mecsas.6 IFN-γ, interferon-gamma.

**BIOFILMS**

*K. pneumoniae* is able to form biofilms, that is, aggregates in which cells that are embedded within a self-produced matrix of extracellular polymeric substance adhere to each other and/or to a surface. Extracellular polymeric substance is a complex structure comprising polysaccharides, proteins and DNA. The most clinically significant *K. pneumoniae* biofilms are those formed on the inner surfaces of catheters and other indwelling devices. *K. pneumoniae* biofilms may also contribute to colonization of the gastrointestinal, respiratory and urinary tract and the development of invasive infections especially in immunocompromised patients.

Development of *K. pneumoniae* biofilms on solid surfaces proceeds from cell adherence, to formation of microcolonies,
maturation and finally dispersal of free-living cells. The most important surface structures involved in the formation process are the type 3 fimbiae and the CP.14 Fimbriae mediate stable adherence, whereas CPs ultimately affect cell-to-cell communication and biofilm architecture. Given the dynamic process of biofilm production and the variability of environmental stimuli, embedded cells must be capable of swift and extensive changes in gene expression. Transcriptional regulation is controlled by quorum sensing, that is, a system of signals and responses that coordinate gene expression in a microorganism community. Putative quorum sensing-associated regulators and autoinducers have been described in K. pneumoniae,14 but available data are still incomplete.

K. pneumoniae cells within biofilms are partially protected from immune defenses. The matrix blocks the access of antibodies and antibacterial peptides and reduces the efficiency of complement and phagocytosis. Existence of mechanisms that actively skew immunity toward reduced inflammatory responses and establishment of chronic infection is also possible.15

What biofilms are most notorious for is high-level resistance to antibiotics. The most important factor determining resistance is bacterial growth status. Within the “inner core” of a biofilm, bacteria are adapted to starvation and low oxygen resulting in growth arrest that, in turn, diminishes the efficiency of antibiotics targeting metabolically active and dividing cells.

ANTIMICROBIAL RESISTANCE

K. pneumoniae strains are naturally resistant to ampicillin, carbenicillin and ticarcillin because of production of a chromosomal penicillinase, sulfhydryl variable (SHV-1). What K. pneumoniae is notorious for, however, is its propensity to collect resistance plasmids. During the 1980s, K. pneumoniae became the index species for plasmids-encoding extended-spectrum-β-lactamases (ESBLs) conferring resistance to expanded-spectrum cephalosporins. Initially, those were Temoniera (TEM)- and SHV-type ESBLs and coexisted on the plasmids with elements encoding resistance to amikoglycides, tetracyclines and trimethoprim-sulfamethoxazole. The 1990s marked the emergence of a new ESBL family, the cefotaximase-M (CTX-M) group, which are currently the dominant ESBLs in K. pneumoniae. These enzymes confer resistance to penicillins and expanded-spectrum cephalosporins but are ineffective against carbapenems. Notably, ESBL-producing organisms have disseminated in the pediatric population as well. A recent study from Italy found increasing rates of intestinal colonization with multiple sequence types (STs) of ESBL in the neonatal intensive care unit, a recognized risk factor for development of infection.16 In another study from the United States that analyzed resistance trends in Enterobacteriaceae in children from 1999 to 2011, the prevalence of isolates exhibiting resistance to third generation cephalosporins increased from 1.39% in 1999–2001 to 3% in 2010–2011.17 Of note, although the K. pneumoniae isolates included in the analysis were 7.7% of the total bacterial population examined, they represented 33.1% of ESBL-producing Enterobacteriaceae. Similarly with adult patients, in children, bloodstream infections (BSIs) caused by ESBL-producing Enterobacteriaceae are associated with prolonged hospitalization and worse outcomes.

The dubious clinical efficacy of penicillin-inhibitor combinations and the fact that a meaningful proportion of such isolates have developed resistance to fluorquinolones and other antibiotics rendered carbapenems the first-line treatment against ESBL-K. pneumoniae infections. The extensive use of carbapenems had as a consequence the emergence and rapid dissemination of MDR, carbapenemase-producing K. pneumoniae (CP-Kp) strains. The prevalent carbapenemase types in K. pneumoniae are the Klebsiella pneumoniae carbapenemases (KPCs) (KPC-2 to KPC-13), the metallo-β-lactamases (Verona integron-encoded metallo-β-lactamase, VIM; imipenemase, IMP; and New Delhi metallo-β-lactamase, NDM types) and the oxacillinase (OXA)-type enzymes (mainly OXA-48). The carbapenemase-encoding genes are usually carried on MDR transmissible plasmids that confer resistance to multiple antibiotics.18 Three clones currently dominate the pandemic spread of CP-Kps: ST258, ST147 and ST15. Studies from different countries show that hospitalized populations, including patients from neonatal intensive care units, are rapidly and persistently colonized with K. pneumoniae ST258, especially in settings where the infection control practices are inadequate. The majority of reports on the dissemination of CP-Kp among children have been published from South-East Asia and Western Pacific Regions.19-21 Data from pediatric patients in Europe, South and North America generally reflect the distribution of carbapenemase-producing Enterobacteriaceae in adults.22-24 In the pediatric population, CP-Kp affect mainly critically ill children with hematologic/oncologic conditions and an immunosuppressive state. The most frequent types of infections are bacteremia followed by respiratory and urinary tract infections.

Virtually, all CP-Kp isolates exhibit extensive drug-resistance phenotypes, and our treatment options are limited.25,26 Polymyxins (colistin and polymyxin B) are considered among the most active agents against CP-Kp. To timely achieve the desired serum concentration, a loading dose of colistin is required followed by adequate maintenance doses to attain optimal drug exposure.27 Aminoglycosides (gentamicin or amikacin) demonstrate good susceptibility profile to a proportion of CP-Kp, and this class of agents could serve as a backbone in the treatment of CP-Kp infections, provided we exploit their well-studied pharmacokinetic/pharmacodynamic (PK/PD) features and dose them optimally. Treatment with tigecycline is another option, but standard dosing regimens of this agent attain suboptimal exposure to the drug; this could be associated with adverse outcomes when it is used in nonapproved indications, particularly in patients with hospital acquired pneumonia/ventilator associated pneumonia (HAP/VAP) or BSIs. Moreover, this agent may cause permanent teeth discoloration and enamel hypoplasia in children <8 years of age, and its use should be limited only in those children in whom no other treatment option is available.26 Given that fosfomycin displays good in vitro activity against most CP-Kp, this agent could be selected as salvage therapy to treat critically ill patients with infections caused by these pathogens. The potential of fosfomycin to rapidly select resistant mutants during therapy is a matter of concern and this agent should be administered always with another active compound. Although it sounds paradoxical, a proportion of CP-Kp, depending on the geographic region and the type of carbapenemase, exhibits its low carbapenem minimal inhibitory concentration (MICs) (1–8 mg/L), and in patients infected with such isolates, a high-dose/prolonged-infusion regimen of meropenem could drive the PK/PD profile of the drug to acceptable exposure. Finally, the newly approved agent, ceftazidime/avibactam demonstrates good in vitro activity against CP-Kp isolates that produce KPC and OXA-48 enzymes, but not metallo-β-lactamases such as NDM, VIM or IMP. Nevertheless, clinical data on efficacy of ceftazidime–avibactam on infections caused by CP-Kp are scarce.

Despite the limitations in current evidence to guide treatment strategies against CP-Kp infections, as no randomized clinical trials have been performed to this date with this objective, combination therapy appears to hold the most promise. Of note, the positive impact of combination therapy on survival is more apparent in critically ill patients with severe infections.28,29 In low-risk BSIs and in nonbacteremic intra-abdominal or urinary tract infections, combination schemes do not seem to provide any therapeutic advantage over monotherapy.30 Carbapenems
may be one of the core agents in the combination schemes, provided that: (1) the MIC of meropenem is ≤8 mg/L and (2) a high-dose/prolonged infusion regimen is administered. In addition, the triple drug combination of meropenem plus colistin plus tigecycline has been reported as an independent predictor of survival in adult patients with KPC K. pneumoniae BSIs. It should be emphasized that in certain infections, among the aforementioned agents, 1 agent may be preferable over the other because of favorable PK/PD characteristics of the drug for the particular site of infection.

Although characterizing the rapid changes of the K. pneumoniae populations in the last decades as “evolution in real time” would be an exaggeration, these are really impressive: worldwide spread of high-risk clones, ongoing build-up of resistance and re-emergence of hypervirulent strains in the community. Clinicians must realize that K. pneumoniae should be considered a major pathogen now and in the foreseeable future.

REFERENCES


