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# *Klebsiella*: a long way to go towards understanding this enigmatic jet-setter

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## Abstract

*Klebsiella pneumoniae* is the causative agent of a variety of diseases, including pneumonia, urinary tract infections, septicemia, and the recently recognized pyogenic liver abscesses (PLA). Renewed efforts to identify and understand the bacterial determinants required to cause disease have come about because of the worldwide increase in the isolation of strains resistant to a broad spectrum of antibiotics. The recent increased isolation of carbapenem-resistant strains further reduces the available treatment options. The rapid geographic spread of the resistant isolates and the spread to other pathogens are of particular concern. For many years, the best characterized virulence determinants were capsule, lipopolysaccharide, siderophores, and types 1 and 3 fimbriae. Recent efforts to expand this list include *in vivo* screens and whole-genome sequencing. However, we still know little about how this bacterium is able to cause disease. Some recent clonal analyses of *K. pneumoniae* strains indicate that there are distinct clonal groups, some of which may be associated with specific disease syndromes. However, what makes one clonal group more virulent and what changes the disease pattern are not yet clear and remain important questions for the future.

## Introduction

*K. pneumoniae* is well known as a causative agent of both community and nosocomial pneumonia, bacteremia, and urinary tract infections (UTIs). The rise in the number of vulnerable populations, appearance of new syndromes, and rise in antibiotic resistance adds urgency to our need to better understand this pathogen. Despite renewed interest in the past decade, more questions have been raised than answered. Genomic sequence analysis has not revealed virulence factors that typify Gram-negative pathogens, such as specialized secretion systems (types III, IV, and V) for the export of effectors or obvious toxins. So what have we learned and what does this tell us about what we need to learn?

### *Emerging syndromes*

The past three decades have ushered in a new *Klebsiella*-associated syndrome (KAS)— PLAs—and, likely owing to the dissemination of bacteria from PLA infection sites, other metastatic complications (for example, endophthalmitis, meningitis, and necrotizing meningitis) have emerged [1–5]. Unique cases of liver abscesses, sometimes referred to as *Klebsiella* invasive syndrome, were reported in the early 1980s in Taiwan [6,7]; this syndrome includes hepatic infection with *Klebsiella* along with dissemination to other sites. *Klebsiella* is now the primary cause of PLA in Hong Kong, Singapore, Korea, and Taiwan [7]. PLA is no longer limited to Asian geographic regions nor is it restricted to individuals of Asian descent as cases have been reported in non-Asian countries [2,8–10]. Although the reasons for the higher prevalence of *Klebsiella*-associated liver abscesses in Asian countries are unclear, studies suggest that environmental factors in the Southeast Asia region play a role in colonization of the gastrointestinal (GI) tract, which is likely associated with disease [11]. Clinical isolates from KAS have a hypermucoviscosity compared with environmental and other clinical isolates. This phenotype appears to

be required to cause liver abscesses in a mouse model for this disease [12].

Endogenous endophthalmitis (EE) secondary to *Klebsiella* liver infection (KPEE) is also an increasing concern [13,14]. The prevalence of KPEE among EE cases appears to be higher in Asian countries than in non-Asian countries [15]. Whether this is because a liver abscess is the source for the majority of cases in Asia (whereas it is endocarditis in the United States [US]) or there are other factors is still unknown [16]. As with liver abscesses, the primary risk factors associated with the development of KPEE are diabetes mellitus and liver disease [17]. Given that the total number of people with diabetes is projected to rise to nearly 366 million by the year 2030, it is reasonable to expect the number of *K. pneumoniae* cases to reflect this increase [18].

Although there have been several studies highlighting and investigating *Klebsiella* liver abscesses and resulting complications, an understudied yet severely affected population consists of burn injury patients [19]. Infection is a common cause of death in these patients [20]. Bacterial identification records at the US Army Institute of Surgical Research Burn Center indicate that *K. pneumoniae* is one of the most common isolates recovered post-admission. Importantly, this timing suggests nosocomial transmission and calls attention to the need for improved healthcare setting practices [21].

*Klebsiella* pneumonia and subsequent septicemia in developed countries are now most commonly associated with nosocomial infections and with long-term nursing facilities [22-26]. Furthermore, there have been reports of drug-resistant *Klebsiella* outbreaks in these facilities [27,28]. Whereas only 5% of acute-care hospitals reported at least one carbapenem-resistant *Enterobacteriaceae* (CRE) case, 18% of long-term acute care facilities reported CRE infections in 2012 [29]. Additionally, both the carriage and the incidence of *Klebsiella* infections increase with age [30,31]. With an increase in the average age of our populations, we can expect an increase in these types of infections if we do not implement changes in our ability to control *Klebsiella* in the environment or if we do not develop improved treatments.

Of the many species within the genus *Klebsiella*, *K. pneumoniae* is considered the most medically important *Klebsiella* species, causing 75% to 86% of clinical *Klebsiella* infections, with the next most commonly recovered species being *K. oxytoca*, which accounts for 13% to 25% of infections [32-34]. Determination of the exact contribution of various *Klebsiella* species to disease in humans is made difficult by the fact that clinical labs rarely speciate *Klebsiella* isolates. In an environmental study of the incidence of *Klebsiella* species in surface waters, it was found that in over half of the samples tested, *K. pneumoniae* was the predominant species. The same group determined that the virulence capabilities (as measured by siderophore production, serum resistance, and fimbriae presence) of the "environmental" *K. pneumoniae* isolates appear to be similar to those of "clinical" *K. pneumoniae* isolates [35,36]. Of notable difference was the relative absence of characterized virulence factors in the less medically prevalent, or "environmental", species: *K. planticola* and *K. terrigena*. These two "environmental" species were recently renamed *Raoultella planticola* and *R. terrigena*, respectively [37], but the new designations are not always used. Whether the distribution of various *Klebsiella* species or the presence of virulence capabilities in individual strains is responsible for *K. pneumonia* being the predominant clinical species is still unclear.

Interestingly, similar *Klebsiella* species distribution is seen in *Klebsiella* animal clinical isolates where *K. pneumoniae* is the predominant species. As in humans, infections of the respiratory tract, urinary tract, sepsis, and abscesses are the most common infection types [38,39]. *K. pneumoniae* is also of particular concern in dairy farms as it causes mastitis and leads to loss of milk production and death of affected livestock [40–42]. Additionally, drug-resistant strains and evidence of horizontal transfer have been reported in animals [43,44]. This is of concern for the agriculture industry and for domestic animals as extended-spectrum β-lactamase (ESBL)-producing strains have been isolated in companion animals and may serve as a reservoir for drug-resistant strains [45–47]. For more on resistance mechanisms, see the "*Klebsiella* and antibiotic resistance" section below.

#### *Lessons learned from genome sequencing*

*Klebsiella* can be isolated from the environment as well as from the clinical setting as the causative agent of disease. However, what is not clear is what makes a strain a "clinical" strain versus an "environmental" isolate nor is it known what makes KAS isolates fundamentally different from other clinical isolates. Whole genome sequencing has provided more questions than answers. A genomic comparison of six *Klebsiella* strains—four clinical isolates, an environmental saprophyte, and a human commensal—showed a core set of 3,631 genes, or about 65% to 75% of the genomes, indicating a large amount of variability between strains [48]. A second genomic study between four strains drew many of the same conclusions and also identified 13 regions of genome plasticity containing transposases or phage genes [49]. Recently, an intermediate strain was identified that possess metabolic pathway genes previously

found only in nitrogen fixers or in clinical isolates, in addition to two novel antibiotic resistance genes [50].

Although some differences, generally in metabolic capabilities, have been seen between clinical isolates and environmental nitrogen-fixing saprophytes, many features are the same between these groups, and there is enough interstrain variability to make prediction of key virulence determinants difficult [51]. However, a recent large-scale study analyzing capsule type, metabolic activity, and sequence diversity of several housekeeping genes has begun to shed some light [51]. This study showed evidence of a significant genetic exchange between *Klebsiella* isolates and horizontal transfer of *cps* (capsule) loci such that the same capsule type (K-type) is associated with different clonal groups. Together, these data suggest that K-type alone may not be the best predictor of virulence. These studies also suggest the continued evolution of the species to fill environmental niches while maximizing pathogenic potential. Sequencing of additional isolates from different settings combined with the testing of isolates in the various models of disease should help to make correlations between genetic content and pathogenic capability.

### *Recent discoveries and new characterizations of previously known virulence factors*

Capsule, fimbriae, lipopolysaccharide (LPS), siderophores, urease, and efflux pumps represent the short list of determinants in which the contribution to disease progression has been studied and clearly demonstrated [23,52]. Advances in our understanding of some of these virulence determinants have progressed in recent years, but the identification of additional virulence determinants has been more difficult. The sequencing of clinical isolates has failed to identify any virulence factors—such as type III secretion systems or exotoxins—that specifically target a host during infection [53-55]. Determinants found to be important for virulence in the host also may play a role in an environmental setting and are present in a sequenced saprophyte [56]. Modification of these factors, or their expression, that allows better survival in a host and aids in the transmission between hosts may represent an adaptation of *Klebsiella* to a new environment.

The majority of clinical isolates express a robust polysaccharide capsule. Although many isoforms exist, clinical isolates are often of the K1 or K2 variety [51,57,58], but this may not reflect an inherent contribution of these capsule types to virulence versus a clonal association of these *cps* loci with other genes that contribute to virulence [51]. Clearly, though, capsules are the most significant virulence determinant of *Klebsiella* [59,60]. In mouse models of pneumonia, the capsule is absolutely required to establish colonization and disease [59]. A capsule likely inhibits the phagocytic activity of innate immune cells [61–65] and may aid in environmental survival by resisting desiccation. Additionally, a capsule may provide some degree of serum resistance by preventing complement factors from accessing the bacterial membranes [66]. The importance of capsule in colonization of the GI tract may be strain-specific, as some non-capsulated mutants have been shown to survive in the GI tract as well as wildtype strains [67], whereas a capsule mutant of a different genetic background was defective in colonization [63].

Underlying the importance of capsule, an ever-growing list of transcriptional regulators of capsule gene expression has been identified [68–72]. Of particular note are MagA, RmpA, and RcsAB [69,71,73]. Whereas classic clinical strains of *Klebsiella* produce a capsule necessary for the infection of patients with long-term comorbidities, new hypervirulent strains associated with liver abscesses and capable of causing disease in young, healthy individuals have emerged [74]. These strains are often associated with a hypermucoviscous phenotype caused by an RmpA-mediated increase in capsule production [75]. Although several studies of PLA have demonstrated that a lack of the hypermucoviscous phenotype is correlated with a lack of disease, these studies used strains with mutations in the regulators of the capsule, including *rmpA* and *magA* but did not directly test the need for the capsule by deleting the capsule synthesis genes themselves [70–72]. Furthermore, clonal analysis of more than 100 strains did not find an association of *magA* with PLA, suggesting that the presence of capsule along with clonal background may be more important than the presence of *magA* [51].

As with many pathogens, *Klebsiella* strains can express a variety of iron acquisition systems, including the enterobactin, aerobactin, and yersiniabactin siderophore systems, as well as hemin and transferrin transporters. The contribution of aerobactin and yersiniabactin during infection has been clearly demonstrated, whereas enterobactin seems to be dispensable in some animal models [23,76,77]. However, lipocalin 2 was shown to inhibit enterobactin-dependent iron acquisition and bacterial growth in the perivascular spaces of the lung during pneumonia [78,79]. This bacteriostatic action preventing the spread of infection indicates that enterobactin may be necessary for penetration to deeper tissues. This same group also demonstrated that yersiniabactin allows the growth of bacteria in the airways but is inhibited by transferrin in the serum in deeper tissues [78]. Together, these data indicate that various iron acquisition systems are needed to overcome host defenses in different anatomical compartments.

To establish infection, *Klebsiella* must first colonize the host, and it has been hypothesized that fimbriae, particularly type 1 and type 3, assist in urinary and possibly upper respiratory tract infections by allowing for the attachment of the bacterium to the surface of indwelling devices and host cells, preventing clearance [23]. Studies of the two fimbriae classes have shown that neither type 1 nor type 3 fimbriae are necessary for GI tract colonization or lung infection but that type 1 fimbriae were required for UTI in mice [80,81]. Type 3 fimbriae, however, were shown to promote biofilm formation [82]. A role for these two fimbriae classes was recently demonstrated *in vivo* by using a UTI model that includes an implanted device mimicking a catheter. Both type 1 and type 3 fimbriae were necessary for the efficient colonization of the urinary tract when a catheter is present, allowing persistence in the bladder as well as attachment to the implanted device [83].

With increased recognition of *Klebsiella* as a pathogen and the increased isolation of strains resistant to a wide variety of antibiotics, several different approaches have been used in a variety of mouse models of infection in an attempt to identify additional virulence factors. A transposon insertion screen of *Klebsiella* in both a model of GI colonization after streptomycin treatment and a model of UTI, LPS, and fatty acid synthesis genes and genes related to metabolism and protein expression were identified. In the UTI model, this screen did identify genes related to bacterial adhesion [84]. A second GI screen using ampicillin treatment identified a different complement of metabolic genes and transcriptional regulators [85]. Although this difference was attributed to the different antibiotics used to facilitate the establishment of *Klebsiella* in the GI tract, it also may be due to bacterial strain variation or differences in the mouse genetic background. A signature-tagged mutagenesis screen using a model of acute pneumonia identified a number of genes of potential importance during this mode of infection, including a number of metabolic factors, cell surface and membrane components, transporters, and regulators in addition to many hypothetical genes [59]. However, many of these genes did not have virulence defects when tested in competitive index or single infections in the same mouse model. A list of similar classes of genes was developed by using an oral infection model of PLA [70] and a *Dictyostelium* model of phagocytosis [86].

With all of these screens, it is interesting to note a general lack of overlap of identified genes. This may be due in part to the fact that none of these screens was saturating and thus only a limited number of genes were identified in each screen. This also could be indicative of mechanisms that compensate for the loss of individual genes, different infection models used, or different strain backgrounds (both pathogen and host). A general lack of follow-up on many novel factors suggests that the genes identified either were not pursued or did not bear out as important when studied in depth.

## *Klebsiella* and antibiotic resistance

The urgency to better understand *Klebsiella*-host interactions is on the rise because of changes in antibiotic resistance patterns of clinical isolates. β-lactam drugs have been the choice for treating *Klebsiella* infections because of the presence of aminoglycoside-modifying enzymes, macrolide esterases, and efflux systems that render many other drug classes ineffective. However, the use of β-lactams has become difficult in recent years as various classes of β-lactamases have been identified and found in clinical *Klebsiella* isolates. The incidence of carbapenem resistance in *Klebsiella* is rapidly increasing, rising from 1.6% to 10.4% between 2001 and 2011 [29]. This increase in CRE has caused the Centers for Disease Control and Prevention to raise an alarm, listing CREs and specifically *Klebsiella* as an urgent threat to public health requiring immediate and aggressive action [87].

The first Gram-negative chromosomally encoded β-lactamase was discovered in 1940 in an *Escherichia coli* strain [88]. Twenty-five years later, the first plasmid-encoded β-lactamase, TEM-1, was identified, also in *E. coli* [89]. A sulfhydryl variant of TEM termed SHV-1 appeared in *Klebsiella* as well as in *E. coli* shortly thereafter [90,91]. The ability of later variants of SHV-1 to hydrolyze a larger spectrum of β-lactam drugs, including oxyimino-cephalosporins, led to these enzymes being designated ESBLs [92]. SHV-18, the variant of this lineage of β-lactamase commonly found in the clinic today, was first isolated in 2000 and is encoded on a plasmid that was readily transferred to *E. coli* in culture [93]. Despite the extended spectrum of hydrolysis by these enzymes, carbapenems were still viewed as the first-line drug of choice for treating *Klebsiella* infections. This began to change in 2001 with the publication of the first carbapenem-resistant *K. pneumoniae* (CRKP) strain, isolated in North Carolina in 1996 [94]. The enzyme responsible for this increased degree of resistance, named *K. pneumoniae* carbapenemase (KPC-1), was identified during surveillance testing in 2000. KPCs are β-lactamases whose active site allows acylation of most carbapenems [95]. This initially was considered an isolated case as no other carbapenemase-producing *Klebsiella* strains had been isolated in the clinic during the years between the initial collection of the original KPC-1 strain in 1996 and its characterization 4 years later [96,97]. However, it was not long before KPC-2 and its variants appeared and spread, first in the New York area of the US, then throughout Europe, South

America, Israel, and the Far East [96,98,99]. It was later determined that KPC-1 and KPC-2 were identical enzymes, with an error occurring in the original KPC-1 sequence [98].

The problem of carbapenem resistance was compounded by the discovery of the metallo-β-lactamases (MβLs). The Verona integrin-encoded metallo-β-lactamase (VIM) and imipenemase (IMP) are relatively common integrin-encoded carbapenemases that are readily transferred among the *Enterobacteriaceae* [100]. The arrival of the New Delhi metallo-β-lactamase (NDM-1) introduced an enzyme that, like VIM and IMP, can hydrolyze all β-lactams except aztreonam but resides on a readily transferrable plasmid. Highlighting the transferability of these genes, this plasmid also was found in an *E. coli* isolated from the same patient from which NDM-1 *Klebsiella* was isolated [101]. The structural determination of NDM-1 revealed that this enzyme has a large flexible active site with many catalytic residues on flexible loops that allow a large variety of substrates to be coordinated with the zinc-activated water molecule for hydrolysis [102]. This flexibility likely will preclude the use of new β-lactam derivatives using the currently available drug backbones.

A third class of carbapenemases, the oxacillinases (OXAs), has begun to disseminate throughout the world. OXA-48, a clinically significant oxacillinase, is capable of hydrolyzing penicillins and carbapenems but does not function against broad-spectrum cephalosporins [103]. However, isolates carrying the $bla_{\text{OXA-48}}$ gene often possess additional β-lactamase genes that render these cephalosporins unusable [103,104]. For an in-depth review of these major carbapenemases, including details of their geographic spread, see the recent review by Nordmann and Poirel [105].

Specific strains with their associated resistance gene profiles are seen primarily in their endemic regions. However, OXA-48 initially identified in a carbapenem-resistant *K. pneumoniae* strain in Turkey has now been identified in Western Europe from Spain to The Netherlands, North Africa, and the Eastern Mediterranean and most recently in North America, Australia, and China [103,104,106,107]. ST258, a *K. pneumoniae* strain harboring KPC-2, is seen primarily in North America [108], whereas NDM-1-bearing strains are found primarily in the Indian subcontinent. The geographic distribution and substrate range of several clinically relevant β-lactamases are listed in Table 1.

To track the spread of *Klebsiella* strains during an outbreak, multiple methods of typing have been employed. These include multi-locus sequence typing, pulsed-field gel electrophoresis, and ribotyping [109]. Although certain sequence types are more prevalent than others in community or hospital settings, several studies have demonstrated that these strains are not correlated specifically with individual antibiotic resistance genes. ST258, likely derived from ST11, often bears a *kpc* gene conferring carbapenem resistance. The *kpc* gene, however, is contained within the Tn4401 mobilizable element, a Tn3-based transposon, which has been found in other sequence types, and on plasmids of various sizes and incompatibility groups [99,108]. A large study (conducted in Beijing, China) of outbreak strains and their resistance gene profiles showed that outbreaks in the clinic were clonal but that the clones and their particular resistance profiles varied with time and correlated with clinical characteristics of the infected patients. Strains identified in this study included the first OXA-48-bearing strain in China as well as multiple extremely drug-resistant (XDR) and pandrug-resistant (PDR) isolates [106]. There does not seem to be a direct correlation between a particular resistance profile and sequence type. As such, sequence typing is not a good indicator of drug resistance but is useful in tracking strains during an epidemic, allowing the identification and elimination of the source of the outbreak.

The increasing isolation of clones with distinct resistance gene profiles indicates that *Klebsiella* is evolving within its local niche before spreading. As virulence factors that specifically target the host have not been identified, it is possible that antibiotic resistance drives evolution of clinical strains. Clonal isolates have been shown to accumulate only a few single-nucleotide polymorphisms over months in a clinical setting, with most changes to sequences affecting antibiotic, solvent, and metal resistance genes [104]. The degree of resistance to antimicrobial compounds in a single clinical strain can be extensive, as detailed in Table 2. Many of these factors reside on resistance plasmids with broad host range. More than 30 distinct plasmids have been identified in various *K. pneumoniae* isolates with sizes up to 270 kb [110]. These plasmids often carry multiple antibiotic resistance genes, sometimes on transposable elements. For example, Tn1331, a transposon associated with the *Klebsiella* plasmid pJHCMW1, contained genes for an ESBL, oxacillinase, quinolone resistance, and streptomycin and spectinomycin resistance [110].

The ease of worldwide travel is changing the geographic distribution of these resistances [111]. With this spread comes the possibility of resistance gene transfer between various *Klebsiella* strains as well as with other *Enterobacteriaceae*, leading to global expansion of carbapenemase resistance [112]. $bla_{\text{KPC-2}}$ with its Tn4401 transposable element has now been detected in *Enterobacter cloacae*,

**Table 1. β-lactamases commonly found in *Klebsiella* clinical isolates**

| Class | Gene product | | Substrate range | Geographic distribution | References |
|---|---|---|---|---|---|
| | Abbreviated name | Expanded name | | | |
| Ambler class A (serine-β-lactamase) | TEM-1 | Temoneira (patient name) β-lactamase | Primarily penicillins, including ampicillin | Worldwide. Most common plasmid-borne β-lactamase in the *Enterobacteriaceae* | [127] |
| | SHV-18 | Sulfhydryl variant ESBL | Penicillins; oxyimino-β-lactams; aztreonam | Worldwide | [93] |
| | CTX-M | Cefotaxime-hydrolyzing ESBL | Broad-spectrum cephalosporins and monobactams; no activity against cephamycins or carbapenems | Worldwide | [128] |
| | KPC | *Klebsiella pneumoniae* Carbapenemase | Penicillins, cephalosporins; lower but clinically relevant activity against carbapenems, aztreonam; low activity against cephamycins and ceftazidime | Worldwide, especially the US, South America, China, Israel, Greece, and Western Europe | [29,98,112,129,130] |
| Ambler class B (metallo-β-lactamase) | VIM | Verona integron-encoded metallo-β-lactamase | All β-lactamases except monobactams (aztreonam) | Worldwide, especially Asia-Pacific region, including Australia, India, the Philippines, Japan, and China | [95,101,131] |
| | IMP | Imipenemase | | | |
| | NDM | New Delhi metallo-β-lactamase | | Worldwide, especially India, Pakistan, Bangladesh, and the Balkans | |
| Ambler class D (serine-β-lactamase) | OXA-48 | Oxacillinase | Penicillins; carbapenems (low but clinically relevant activity); some narrow-spectrum cephalosporins; no activity against oxyimino-cephalosporins | Turkey, Western Europe, North Africa, and the Eastern Mediterranean | [95,103,132-134] |

Clinically relevant β-lactamases associated with *Klebsiella* are listed by Ambler class with substrate ranges and geographic reservoirs.
Abbreviations: ESBL, extended-spectrum β-lactamase.

**Table 2. Resistance genes encoded by *Klebsiella pneumoniae* BAA-2146**

| Gene | Resistance | Mechanism |
|---|---|---|
| *ant3ia* | Spectinomycin | Aminoglycoside O-nucleotidylyltransferase |
| *aph6id* | Streptomycin | Aminoglycoside O-phosphotransferase |
| *bacA* | Bacitracin | Undecaprenyl pyrophosphate phosphatase |
| $bla_{SHV-2}$ | Penicillins and cephalosporins | Class A β-lactamase |
| $bla_{CTX-M}$ | Penicillins and cephalosporins | Class A β-lactamase |
| $bla_{NDM-1}$ | All β-lactams except aztreonam | Class B β-lactamase |
| $bla_{CMY-2}$ | Narrow-spectrum cephalosporins | Class C β-lactamase |
| *emrD* | Multiple compounds | Major facilitator superfamily (MFS) efflux pump system |
| *qnrB* | Fluoroquinolones | Pentapeptide repeat family |
| *sul1, sul2* | Sulfonamide | Resistant dihydropteroate synthase |
| *tetA* | Tetracycline | Tetracycline efflux pump |
| *acrAB/tolC* | Aminoglycosides, glycylcycline, macrolides, β-lactams, and acriflavin | Resistance-nodulation-division (RND) efflux pump system |
| *ksgA* | Kasugamycin | Dimethylation of 16s rRNA |
| *macAB/tolC* | Macrolides | ATP-binding cassette (ABC) multidrug efflux pump |
| *mdtGHK* | Fosfomycin, norfloxacin, and deoxycholate | MFS efflux pump system |
| *mdtABCD* | Multiple compounds | RND efflux pump system |
| *cmeABC* | Multiple compounds | RND efflux pump system |
| *ydhE* | Quinolones, novobiocin, and others | Multidrug and toxin extrusion (MATE) efflux pump system |
| *fosA* | Fosfomycin | Glutathione-dependent glyoxalase |
| Multiple | Arsenic, cadmium, chromium, cobalt, copper, mercury, and zinc | Heavy metal resistance by efflux pump systems |

Genes for antimicrobial compound and metal resistance were identified by annotation with RAST (Rapid Annotation using Subsystem Technology) and searching the ARDB (Antibiotic Resistance Database). The listing includes substrate specificity and mechanism of action [53,135,136].

*Serratia marcescens*, *Citrobacter freundii*, and *E. coli* [113]. NDM-1 already is found in a number of *Enterobacteriaceae*, including *Salmonella* and *Enterobacter* in addition to *Klebsiella* and *E. coli* [114]. Furthermore, exposure to NDM strains is not limited to transmission in a clinical setting [108], as public water supplies in India and China have detectable levels of NDM-1 in isolates, implying that environmental exposure may be a first contact point [108,115]. This transfer of antibiotic resistance genes has the potential to create extremely drug-resistant strains of species other than *Klebsiella*.

In addition to these β-lactamases, a change in a group of AraC-like regulators typically involved in the stress response is contributing to the evolution of *K. pneumoniae*. *rarA* has been shown to be upregulated, along with homologs *marA* and *soxS*, in multidrug-resistant *Klebsiella* strains isolated from geographically distinct regions. This increase in *rarA* expression results in increased expression of the efflux pump system encoded by *oqxAB* [116]. Furthermore, the upregulation of *rarA*, *marA*, and another homolog, *ramA*, causes increased production of the efflux pump AcrAB in addition to OqxAB, leading to resistance to multiple antibiotics, including tigecycline [117].

Our inability to control the presence and spread of *Klebsiella* in clinical facilities is indicative of our need for novel methods of controlling infection. As *Klebsiella* has not been demonstrated to directly target the host immune response, controlling bacterial growth by limiting metabolism and stopping passive immune evasion by blocking the bacterial stress response may be viable strategies. *Klebsiella* has a large number of transcriptional regulators, likely providing overlapping functionality. This is demonstrated by the presence of *rarA* and *ramA* in *Klebsiella* in addition to the normal complement of *marA* homologs found in other *Proteobacteria*. These regulators may be targets for future therapy, but our understanding of *Klebsiella* metabolism, transcriptional regulation, and stress response first must be improved.

## Source of *Klebsiella* infections

Asymptomatic GI carriers of *Klebsiella* may act as infectious reservoirs. Various studies have shown that 5% to 38% of the general population is colonized without disease, with rates higher in hospital staff. Carriage rates increase up to 77% in hospitalized patients or individuals residing in long-term care facilities, with the degree of colonization proportional to the length of stay [23]. Antibiotic treatment further increases the incidence of *Klebsiella* GI colonization in hospitalized patients [118].

Reservoirs for *Klebsiella* other than GI tract colonization also exist. Persistence of *Klebsiella* in the environment and the need for control of bacterial spread have long been known [118,119]. This was highlighted during the recent outbreak of *Klebsiella* at the National Institutes of Health Clinical Center. The outbreak strain was cultured from six sink basins as well as from a ventilator that had been cleaned once with bleach and twice with a quaternary ammonia compound [120]. Whether these fomites contributed to the spread of this infection could not be established but serves as a reminder that *Klebsiella* can persist throughout a facility and may be resistant to chemical sterilization. This was further demonstrated by the isolation of a *Klebsiella* ST258 isolate that demonstrated high-level resistance to chlorhexidine, a topical antiseptic used in hospitals to decontaminate surfaces as well as for skin sterilization prior to surgical procedures [121]. *Klebsiella* also is present outside the clinic. This is a ubiquitous organism in nature, found on vegetation and surface waters and in soil [122]. Although environmental strains appear to be a distinct clonal group of *Klebsiella* [50], they nevertheless have been shown to be as virulent as clinical isolates in the urinary and GI tracts [123].

This information indicates that control in and out of the clinic is necessary not only to prevent the spread of the bacterium to uninfected patients but also to minimize the transmission of resistance factors to other pathogens. This needs to include active surveillance in clinics, intake testing of patients, and the use of carriage cohorts to isolate individuals shown to carry or be infected with *Klebsiella*. Additionally, environmental surveillance is needed to minimize transmission through communal surfaces, such as sinks and doorknobs, as well as personal fomites, such as white coats, cell phones, neckties, and stethoscopes [124-126].

## A long way to go…

Clearly, an understanding of the bacterial determinants promoting GI colonization, dissemination to other body sites, or the ability to cause disease at specific sites (for example, lung, urinary tract, sepsis, and liver abscess) is still far from complete. Capsule remains a key factor in *Klebsiella* virulence, and strains with mutations or gene acquisitions that increase capsule production may be more virulent. Nevertheless, we do not fully understand the role of capsule, or yet-to-be-defined virulence determinants, in various *Klebsiella* disease syndromes. Current information points to the possibility that clinical strains represent distinct clonal groups relative to environmental strains, yet, so far, there are no clear identifying features that would explain why these clinical strains are found associated with disease but environmental isolates typically are not. Genomic analyses, though still in their infancy for *Klebsiella*, might be helpful in pointing the way towards answers. In this regard, it will be essential to

sequence multiple isolates from diverse geographic locations and from diverse anatomical sites and disease syndromes. Future studies also will need to address whether antibiotic resistance determinants affect virulence in addition to influencing treatment options.

## Abbreviations

*Cps*, capsule; CRE, carbapenem-resistant *Enterobacteriaceae*; EE, endogenous endophthalmitis; ESBL, extended-spectrum β-lactamase; GI, gastrointestinal; IMP, imipenemase; *K. pneumoniae; Klebsiella pneumoniae*; KAS, *Klebsiella*-associated syndrome; KPC, *Klebsiella pneumoniae* carbapenemase; KPEE, endogenous endophthalmitis secondary to *Klebsiella* liver infection; LPS, lipopolysaccharide; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase; PLA, pyogenic liver abscesses; UTI, urinary tract infection; VIM, Verona integrin-encoded metallo-β-lactamase.

## Disclosures

The authors declare that they have no disclosures.

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