# Genomic Epidemiology: Revealing Hidden Reservoirs for *Klebsiella pneumoniae*

**Amee R. Manges**

Faculty of Medicine, School of Population and Public Health, University of British Columbia, Vancouver, Canada

**(See the Major Article by Davis et al on pages 892–9.)**

Classically defined infectious disease epidemiology is being upended by the replacement of low-resolution phenotypic and molecular technologies with high-resolution whole-genome shotgun sequencing of pathogens. The evolution of molecular epidemiology has been under way for 10 years, but the speed of the transition has recently quickened. Previously unrecognized pathogen reservoirs and modes of transmission have been uncovered for diseases for which the epidemiology was considered to be largely solved. For example, traditional hospital-acquired infections such as those caused by *Clostridium difficile* have recently been shown to have a community and environmental reservoir larger than previously thought [1]. Based on whole-genome sequencing (WGS), up to 45% of the *C. difficile* strains causing 1250 infections over a 3.5-year period may have come from nonhospital or environmental reservoirs [1]. Likewise, WGS-based studies of human extraintestinal infections (eg, urinary tract, kidney, and bloodstream infections) have uncovered cryptic pandemics caused by *Escherichia coli*, including *E. coli* O25b:H4 sequence type (ST) 131 [2–4]. Infections caused by this *E. coli* lineage can account for 30% of all extraintestinal infections and an even greater fraction of the antimicrobial-resistant infections [4, 5]. Reservoirs for extraintestinal infections are often unknown, as traceback investigations that link infection to source are more challenging than for traditional foodborne diarrheal infections with shorter "incubation" periods.

Epidemiologic information has always been the gold standard onto which inferences are made about the behavior of infectious diseases in populations. Molecular tools have been used to support the conclusions made from epidemiologic studies and public health investigations. However, there is an important new role for WGS in molecular epidemiology (now fashionably termed "genomic epidemiology"). We are asking this new tool to provide evidence of epidemiologic links between reservoirs or sources and infections when epidemiologic information of sufficient granularity is not available or when exposure or transmission are distant in time or cannot be tracked accurately. Examining genome sequence–level differences can fill the holes when subclinical intestinal colonization with an opportunistic pathogen such as extraintestinal pathogenic *E. coli* or *Klebsiella* species can occur months prior to infection. Deployment of higher-resolution genomic epidemiology tools is beginning to augment our understanding of source, transmission, and risk factors for colonization with opportunistic pathogens.

In the study by Davis et al in this issue of *Clinical Infectious Diseases*, the potential for a food-animal reservoir for *Klebsiella pneumoniae* is investigated. This is an increasingly important question given the rise in multidrug-resistant phenotypes exhibited by human clinical isolates of *K. pneumoniae*. It is imperative to know the reservoirs and to understand the source and circulation of antimicrobial-resistant organisms and their genetic determinants of resistance. *Klebsiella pneumoniae* can cause diverse types of infections, including hospital and community-acquired infections such as urinary tract infections, as well as infections in animals. The environmental reservoirs for *Klebsiella* causing human infections are not well known. The authors postulate that some *K. pneumoniae* may originate from food-animal or retail meat sources; the authors examine this link using phylogenomic methods and an animal model of virulence.

Retail turkey, chicken, and pork meats were sampled from a single community, where clinical samples of blood and urine from human infections were contemporaneously screened for *K. pneumoniae*. Ten percent of the human clinical samples and 47% of the retail meat isolates yielded *K. pneumoniae*. Four groups, as defined by multilocus sequence typing (MLST) and analysis of single-nucleotide polymorphisms within the core genome of these strains, contained members from both human and retail meat sources. Virulence of these closely related *K. pneumoniae* strains was tested in mice, and all groups exhibited relatively low virulence in vivo and were similar by source. Although all members of these 4 groups were antimicrobial susceptible, this does not preclude the possibility that drug-resistant *K. pneumoniae* could be transferred via the foodborne route.

There have been several studies that have identified extended-spectrum β-lactamase (ESBL)–producing *Klebsiella* in food animal and retail meat sources. In retail meat samples (chicken beef and pork) from the Netherlands, 7.7% contained ESBL-producing *Klebsiella* species, and all were identified in chicken meat samples [6], whereas 20% of rectal swabs from hospitalized patients were positive for ESBL-producing *Klebsiella* in the same region. MLST results for these human and retail meat isolates suggested that related *Klebsiella* species were present in both human and food animal sources [6, 7]. The prevalence of ESBL-producing *K. pneumoniae* in healthy chickens in Japan was estimated to be 3% [8]; 36% of respiratory infections were caused by *K. pneumoniae* in swine in China, and nearly 100% of these isolates were ESBL producers and 69% were fluoroquinolone resistant [9]. Plasmids carrying multiple antimicrobial resistance determinants, including ESBL genes, can spread widely within the Enterobacteriaceae and can cause epidemics in food animals [10]. The prevalence and distribution of multidrug-resistant *Klebsiella* varies widely in food-animal reservoirs, indicating

that the extent of *Klebsiella* transmission from food animals to humans may vary by geographic region. In the future, collecting epidemiologic information on the consumption or handling of specific retail meat products on subjects with drug-resistant or genetically related *K. pneumoniae* would enhance the phylogenomic-based conclusions reached by Davis and colleagues.

It is interesting to note that of the 5 genetically related clusters identified, 2 groups included turkey, 2 groups included pork, and 1 group included chicken isolates. As all retail meat commodities yielded *K. pneumoniae* in this initial study, it may be important to include samples from retail beef meat or beef cattle as part of future investigations just to rule out the presence of human-associated *K. pneumoniae*. Although not mentioned by Davis et al, the study also identified multiple other clusters of genetically related strains, comprising either all human or all animal isolate members. For example 6 closely related ST380 strains were identified from among the human clinical isolates (Figure 2 of Davis et al); all 6 were sampled within a 4-month period and at least 2 were recovered from community-acquired infections, including 1 from a 23-year-old subject. If there were no obvious relationships among individuals with *K. pneumoniae* ST380, such as residence in the same healthcare institution, then this observation is further evidence that a common reservoir for this ST380 strain may exist. Similarly, 2 indistinguishable ST1688 *K. pneumoniae* strains were identified from 2 patients with community-acquired infections presenting 2 months apart. Common lineages of *K. pneumoniae*, including 3 closely related ST1694 and 2 closely related ST1693 isolates, were identified in pork and chicken meat, respectively. Identification of retail meat samples contaminated with genetically related isolates also points to the possibility that a food-animal reservoir may contribute to the dissemination of *Klebsiella*.

Evidence for the clonal expansion and dissemination of certain ESBL-producing

*E. coli* causing extraintestinal infections is accumulating, as is the link, in some instances, to poultry. It is not too far a stretch to imagine that *Klebsiella* dissemination may operate in a similar fashion. Although common multidrug-resistant *K. pneumoniae* was not recovered from both human and retail meat samples in this study, similar lineages of susceptible isolates were identified from both sources, suggesting food animals may be a reservoir. Not surprisingly, the molecular epidemiology of *K. pneumoniae* has focused on the emergence of specific clones in human extraintestinal infections alone, whereas environmental reservoirs for these organisms have rarely been explored. This study is an example of how WGS and genomic epidemiology can be used to uncover previously unrecognized sources and reservoirs of pathogens causing extraintestinal infections. When colonization and infection are separated in time, reservoirs are undefined, epidemiologic data are incomplete, and chains of transmission are unknown or sparse, genomic epidemiology will be there to fill these data gaps.

## Note

## References

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