**REVIEW ARTICLE**

**Klebsiella, a Hitherto Underappreciated Zoonotic Pathogen of Importance to One Health: A Short Review**

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**Abstract**

Members of the genus, *Klebsiella*, are becoming increasingly challenging to control due to the recent convergence of multidrug resistant (MDR) and hypervirulent (hv) phenotypes in some species of concern to One Health. This short review will provide an introduction to this bacterial genus in the hospital and other settings, update *Klebsiella* taxonomy, and comment on recent findings describing the prevalence of *Klebsiella* species in the food chain, a hitherto infrequently recognised ecologic niche. The paper will also consider this bacterium in the context of the One Health paradigm and its importance to food safety and security.

**Key words:** *Klebsiella*, taxonomy, food safety, antimicrobial resistance, One Health

**INTRODUCTION TO KLEBSIELLA IN THE HOSPITAL AND OTHER ENVIRONMENTAL NICHELIES**

*Klebsiella pneumoniae*, the type species of *Klebsiella*, was first described in 1882 by Carl Friedländer as an encapsulated bacillus responsible for pulmonary infections. The bacterium, *K. pneumoniae*, is a Gram-negative, non-motile member of the *Enterobacteriaceae* family and is epidemiologically linked to nosocomial and community-acquired (CA) infections. Nosocomial or hospital-acquired (HA) *K. pneumoniae* is responsible for 3%-8% of all recorded bacterial infections [1] with a higher mortality rate than CA-*K. pneumoniae* (CA-Kp). HA-*K. pneumoniae* (HA-Kp) harbours more antimicrobial resistance (AMR) genotypes when compared to CA-Kp. In contrast, CA-Kp isolates have a higher prevalence of virulence factor genotypes, such as *rmpA2*, which encodes capsule regulator genes [2].

In contrast to its recognised threat to human health as a nosocomial risk, *Klebsiella* species are ubiquitous in nature and found in ecologic niches, such as plants and animals. Although *K. pneumoniae* has been extensively studied in clinical settings, its role as a hitherto unrecognised zoonosis has been overlooked. An important agent of disease in animals, especially when expressing the hypermucoid phenotype, as observed in California sea lions [3] and African green monkeys [4], *K. pneumoniae* resides in the gastrointestinal tract of domestic/wild animals and humans alike, and colonises foods, such as various meats. This pathogen is also an important aetiological agent in clinical cases of mastitis [5]. Environmental niches include water (drinking and surface), waste effluent (municipal sewage and industrial), and vegetation (plants and soil; Bagley [6]). Some species of bats have

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been reported to carry extended-spectrum  ß-lactamase (ESBL) and carbapenemase-producing K. pneumoniae (Table 1). The ease with which K. pneumoniae acquires and transfers AMR-encoding genes (ARGs) across various niches via horizontal gene transfer (HGT) mediated by mobile genetic elements (MGEs) has led to K. pneumoniae being likened to a “canary in the coal mine” [8,19].

Together with studies reporting similarities at the genomic level between clinical and environmental isolates of K. pneumoniae, especially in relation to virulence factors [27-29], it may now be necessary to re-evaluate K. pneumoniae and the broader Klebsiella genus as a hitherto unrecognised zoonosis (Table 2). This bacterium could be considered in the context of One Health by studying the impact of K. pneumoniae on the health of humans, animals, and the environment. The One Health paradigm is a global public health strategy with three axes (human, animal, and environment). The quintessential One Health issue that affects this triad is AMR. Emergence of ARGs can arise following long exposure periods and sub-therapeutic usage of antibiotics in animals, with dissemination of these ARGs to human pathogens or the gut microbiota through food, the environment, and other routes. In addition, human and animal antibiotics are largely similar, allowing for selection and the subsequent transfer of AMR genotypes between humans and animals, either directly or via environmental routes [30].

CURRENT TAXONOMY OF THE GENUS, KLEBSIELLA

Understanding the taxonomy of the genus, Klebsiella, is important to enable accurate identification and description of the epidemiology of these opportunistic pathogens. Whole genome sequencing (WGS) and more advanced biochemical techniques have facilitated clarification of the taxonomy of Klebsiella. Two major subdivisions of Klebsiella are now recognised and denoted Klebsiella pneumoniae species complex [KpSC] and Klebsiella oxytoca species complex [KoSC] [7,31] (Fig 1). WGS-based analysis divided K. pneumoniae into 7 phylogroups, consisting of 5 species with a 95%-96% shared average nucleotide identity (ANI). One of these phylogroups, Klebsiella pneumoniae sensu stricto, was designated Kp1; the remaining phylogroups of KpSC include K. quasipneumoniae subsp. quasipneumoniae (Kp2), K. variicola subsp. variicola (Kp3), K. quasipneumoniae subsp. similipneumoniae (Kp4), K. variicola subsp. tropica (Kp5), K. quasivaricola (Kp6), and K. africana (Kp7)). In total, K. pneumoniae sensu stricto comprises approximately 85% of clinical isolates, and all members of this phylogroup are generally referred to as K. pneumoniae [7]. In addition to K. pneumoniae, other members of the KpSC are opportunistic and virulent bacterial pathogens, such as K. variicola and K. quasipneumoniae subsp. similipneumoniae [32-33]. Furthermore, WGS also showed that KpSC has a 90% shared ANI with the 9 other Klebsiella species, including K. huaxiensis, K. oxytoca, K. michiganensis, K. pasteurii, K. grimontii, K. granulomatis, K. aerogenes, K. spallanzanii, and K. indica [7].

The other major subdivision of the genus (Fig 1) relates to the K. oxytoca species complex (KoSC), which consists of the following 6 species: K. michiganensis [Ko1]; K. oxytoca [Ko2]; K. spallanzanii [Ko3]; K. pasteurii [Ko4]; K. grimontii [Ko6]; and K. huaxiensis [Ko8] [31]. These 9 phylogroups are assigned on the basis of variations identified in the sequences of a beta-lactamase-encoding gene (bla_OXY). Phylogroups Ko5, Ko7, and Ko9 do not represent a unique species; Ko7 is considered to be a sub-group of Ko6 [22]. Although members of KoSC are less intensively studied, K. oxytoca is the second most likely member of the genus to cause clinical disease after K. pneumoniae [34]. K. oxytoca is an important pathogen and is responsible for antibiotic-associated haemorrhagic colitis (AAHC). Like K. pneumoniae, however, isolates of K. oxytoca have been reported with carbapenem resistance and some shared virulence factors, as discussed below [35]. Representative isolates from the KoSC complex have also been isolated from environmental and animal sources [22,36].

KLEBSIELLA SPECIES CULTURED FROM ANIMALS AND ACROSS THE FOOD CHAIN

The food chain has not traditionally been considered to be a risk factor for transmission of Klebsiella. Despite this, several studies in the recent past have reported isolation of Klebsiella from food-producing animals and food products derived from animals, including milk, salads, and poultry.

Colonisation of domesticated and food-producing/farm animals by Klebsiella species may pose a risk to humans. Co-habiting humans and their companion dogs have been reported to share K. pneumoniae clonal lineages, with some sequence types (STs) identified in dogs having been previously isolated from human infections. Fecal colonisation of dogs by K. pneumoniae was recorded at 39% in one study [37]. A corollary study by the same authors [38] concluded that 60% of K. pneumoniae cultured from companion animals belonged to ST15, which is commonly found in isolates associated with CA- and HA-Kp infections. The majority of these isolates belonged to two PFGE clusters (pulsotypes), which also included human STs.

Contamination of the environment with faeces is another route by which Klebsiella can be transmitted. The faecal-oral cycle was assessed in three dairy farms. The data reported 67% of faecal samples, all of the rumen samples and 89% of water samples obtained were positive for Klebsiella species. Among the genus species detected, K. pneumoniae was the most prevalent in faeces (94%) and rumen (92%), and in swabs obtained from alleyways (100%). Amongst soil and crop samples, K. oxytoca (35% and 7%, respectively), K. variicola (5% and 67%, respectively), and Raoultella planticola (49% and 11%, respectively) were common [39]. Cattle faecal shedding can contaminate the environment and give rise to cases
### TABLE 1: The seven phylogroups within the *K. pneumoniae* species complex, their relevance in cases of human infections, and their detection in other non-human niches.

<table>
<thead>
<tr>
<th>KpSC phylogroup</th>
<th>Klebsiella taxa</th>
<th>Clinical relevance</th>
<th>Zoonotic relevance</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td></td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Animals (e.g., crabs, mussels, dogs, and horses) Environment (e.g., mangroves, water, and lettuce)</td>
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<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>African bush elephant, Brazil&lt;sup&gt;8&lt;/sup&gt;</td>
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<td></td>
<td>Human isolates Brazil&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Human isolates Brazil&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Water, The Netherlands&lt;sup&gt;9&lt;/sup&gt;</td>
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<td>High prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>High prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Amazon river dolphins&lt;sup&gt;10&lt;/sup&gt;</td>
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<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Wild animals (foxes &amp; coatis)</td>
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<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Dogs and elephants&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>Low prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Low prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>High prevalence in food-producing animals (pigs, bovines, &amp; poultry) &amp; in vegetables; lower prevalence in domestic animals and in other environmental sources</td>
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<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Grey-headed flying foxes&lt;sup&gt;2&lt;/sup&gt;</td>
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<td></td>
<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Animals (e.g., <em>Bos indicus</em>), environment (e.g., plants)</td>
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<td>Human isolates Brazil&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Human isolates Brazil&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Food (bananas, Mexico), plants (maize, USA), environment (fungus garden)</td>
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<td>Low prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Low prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Lignin degradation&lt;sup&gt;9&lt;/sup&gt;</td>
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<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>High prevalence in bovine &amp; the environment (soil, rivers/natural ponds, fruits, vegetables, flowering plants, &amp; water catchment). Low prevalence in domestic animals, pigs, &amp; poultry</td>
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<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Nosocomial infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Mosquitos&lt;sup&gt;3&lt;/sup&gt;</td>
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<td></td>
<td>Human isolates Brazil&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Human isolates Brazil&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Pig farm, China&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>Neonatal outbreak China&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Neonatal outbreak China&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Environment (farmland soil, China &amp; Lake Kikker, The Netherlands)&lt;sup&gt;7&lt;/sup&gt;</td>
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<td>Low prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Low prevalence in community &amp; nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Wild animals (birds &amp; anteaters)&lt;sup&gt;10&lt;/sup&gt;</td>
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<td></td>
<td>Low prevalence in Community &amp; Nosocomial isolates Guadeloupe&lt;sup&gt;12&lt;/sup&gt;</td>
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<td>High prevalence in Bovine, and the environment (soil, rivers/natural pond, fruits, vegetables, flowering plants &amp; water catchment). Low prevalence domestic animals, pig, &amp; poultry</td>
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<td><strong>Kp6</strong></td>
<td><em>K. quasivariicola</em></td>
<td>Human gut&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Environment&lt;sup&gt;7&lt;/sup&gt;</td>
<td>Wyres et al. [7]&lt;sup&gt;1&lt;/sup&gt;, Dereeper et al. [13]&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Pig farm, China&lt;sup&gt;6&lt;/sup&gt;</td>
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<td><strong>Kp7</strong></td>
<td><em>K. africana</em></td>
<td>Human gut&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Environment (wastewater, UK)&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Wyres et al. [7]&lt;sup&gt;1&lt;/sup&gt;, Dereeper et al. [13]&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Human infections&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Grey-headed flying foxes&lt;sup&gt;2&lt;/sup&gt;</td>
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</tbody>
</table>

[Superscript numbering shown under the clinical and zoonotic relevance columns refers directly to the references included in the final column of Table 1].
of bovine mastitis, a long-lasting severe intramammary infection that affects milk production. *Klebsiella* mastitis results in a higher culling rate compared to other forms of mastitis, and this bacterium can express AMR phenotypes. The incidence of *Klebsiella* mastitis was also reported to be higher between 2010 and 2020 than between 2000 and 2010 [40].

Cases of septicaemia in neonatal pigs have been attributed to specific *K. pneumoniae* STs, such as ST25, ST278, and ST1978 in Australia and ST25 in England. Pneumonic pigs in Australia were reported to be positive for *Klebsiella* isolates of ST14 and ST17. Both ST14 and ST1978 have been reported in humans in southeast Asia and Europe [41].

TABLE 2 | The nine phylogroups within the *K. oxytoca* species complex, their relevance in cases of human infections, and their detection in other non-human niches.

<table>
<thead>
<tr>
<th>KoSC phylogroup</th>
<th><em>Klebsiella</em> taxa</th>
<th>Clinical relevance</th>
<th>Zoonotic relevance</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ko1 Ko5</td>
<td><em>K. michiganensis</em></td>
<td>ESBL-producing outbreak in a neonatal unit</td>
<td>Environment (baby bath drain, detergent bottles, and milk room)</td>
<td>Chapman et al. [20]³, Chen et al. [21]⁴, Cosic et al. [22]⁵, Merla et al. [23]⁷</td>
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<td></td>
<td><em>K. pneumoniae</em></td>
<td>Preterm gut</td>
<td>Isolated from the environment (food, soil, surface water, and sewage sludge)</td>
<td>Herzog et al. [24], Singh et al. [25], Chapman et al. [20], Cosic et al. [22]⁶</td>
</tr>
<tr>
<td>Ko2</td>
<td><em>K. oxytoca</em></td>
<td>Human gut – pathobiont causing AAHC</td>
<td>Environment (sinks, wastewater drainage systems, washing machines, and medical solutions)</td>
<td>Herzog et al. [24], Singh et al. [25], Chapman et al. [20], Cosic et al. [22]⁶</td>
</tr>
<tr>
<td>Ko3 Ko9</td>
<td><em>K. spallanzanii</em></td>
<td>Isolated from human peritoneal fluid, France and human urine, Italy</td>
<td>Isolated from cow faeces, Italy</td>
<td>Merla et al. [23]⁷</td>
</tr>
<tr>
<td>Ko4</td>
<td><em>K. pasteuri</em></td>
<td>Isolated from a human wound and peritoneal fluid, France and human faeces, Italy</td>
<td>Isolated from sewage sludge and mouse faeces</td>
<td>Cosic et al. [22]⁵, Merla et al. [23]⁷</td>
</tr>
<tr>
<td>Ko6 Ko7</td>
<td><em>K. grimonii</em></td>
<td>Preterm gut</td>
<td>Isolated from a human wound, France</td>
<td>Chen et al. [21]⁴, Cosic et al. [22]⁵, Merla et al. [23]⁷</td>
</tr>
<tr>
<td>Ko8</td>
<td><em>K. huaxiensis</em></td>
<td>Urinary tract infection, China</td>
<td>Isolated from cattle faeces, Italy</td>
<td>Hu et al. [26], Merla et al. [23]⁷</td>
</tr>
</tbody>
</table>

[Superscript numbering shown under the clinical and zoonotic relevance columns refers directly to the references included in the final column of Table 2].

**FIGURE 1** | A schematic listing of the taxonomic division of the genus and the members of the *K. pneumoniae* species complex (KpSC) and the *K. oxytoca* species complex (KoSC).
In addition to the threats posed by *Klebsiella* causing disease in animals, as evidenced by the same STs identified in humans and animals, the bacterium can now be considered to pose a challenge to *One Health*.

**AMR IN KLEBSIELLA SPECIES FROM ANIMALS**

The spread of carbapenem-resistant *Enterobacteriaceae* in the healthcare setting has given rise to concerns about other sectors of the *One Health* paradigm, such as food-producing and companion animals, and the environment. An investigation of carbapenem-resistant *Enterobacteriaceae* (CRE) in poultry in China involved the collection of 739 samples from a range of geographic locations and mammals. Some 55 isolates (7.4%) were identified as carbapenem-resistant *K. pneumoniae* [42]. Further study of these 739 samples in 2019 highlighted 25 *K. pneumoniae* cultured from chicken (n=19), fly (n=5), and dog samples (n=1), and recorded 22 of the 25 samples as positive for New Delhi metallo-β-lactamase (NDM). These 22 *K. pneumoniae* samples were comprised of 6 clinically relevant STs, including ST11, ST13, ST37, ST147, ST256, and ST485, and were associated with carriage of *bla*<sub>NDM</sub>. Phylogenetic analysis suggested that some of these *K. pneumoniae* isolates belonging to ST11, ST37, and ST147 were closely related to human isolates. In addition, the most prevalent plasmid replicon types identified were IncFIB [often found in human and animal ESBL-*K. pneumoniae*], IncX3 [linked to dissemination of *bla*<sub>NDM</sub> amongst the *Enterobacteriaceae*], and IncFII [a carrier of significant ARGs amongst the *Enterobacteriaceae*] [43].

A recent study in China highlighted 38 publications between 2000 and 2020 that reported on *Klebsiella* isolated from cases of bovine mastitis and were shown to be resistant to 9 commonly used antimicrobial agents (sulphonamides and tetracyclines). China has banned the use of these two compounds in animal husbandry, suggesting that these *Klebsiella* species continue to harbour resistance mechanisms [40]. An extensively drug-resistant (XDR) *K. quasipneumoniae* subsp. *similipneumoniae* ST5028 was isolated from a Chinese pig farm [18]. A study involving *Klebsiella* species in Brazil isolated *K. quasipneumoniae* subsp. *similipneumoniae* ST1308 in mosquitoes, which has also been observed in humans. These Brazilian studies also reported *K. pneumoniae* ST11, ST15, and ST340 to be present in humans, animals (dogs, crabs, cats, and pigs) and in the environment (water and man-groves). Isolates of sequence types ST437 and ST198 were found in humans and the environment (water and lettuce). *K. variicola* subsp. *variicola* contains many STs common across animals, humans, and the environment, with ST355 shared between animals [*Bos indicus*] and the environment [plants] [9].

The dissemination of ecologically shared STs expressing AMR phenotypes should be monitored in an effort to develop control strategies to mitigate this occurrence.

**SPREADING OF MANURE ON AGRICULTURAL SOIL AND CONTAMINATION OF THE ENVIRONMENT BY KLEBSIELLA SPECIES**

The spreading of animal manure directly onto farm crops and fields may be a practice that favours the transmission of resistant *Klebsiella* to plants and the broader environment. Additionally, *K. pneumoniae* has endophytic abilities, fixing nitrogen in plants, such as alfalfa, maize, and wheat. Because *Klebsiella* can reside on the stems and leaves of raw produce, *K. pneumoniae* may become ingested, thus causing infections in susceptible individuals [44-46]. Aquatic environments can also become contaminated following effluent discharges from sources, such as industrial settings, farms, hospitals, and municipal sewage. Bivalve molluscs are used in Norway to assess the level of *Klebsiella* species in marine environments and to determine the associated AMR phenotypes. Of 476 samples, 204 were confirmed positive for *Klebsiella* and *Raoultella* using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). These samples included *K. pneumoniae* (n=78), *K. oxytoca* (n=41), *K. variicola* (n=33), *K. aerogenes* (n=1), *R. ornithinolytica* (n=38), and *R. planticola* (n=13). An ESBL-producing *K. pneumoniae* isolate was identified in a marine bivalve growing area that was destined for human consumption, suggesting the food chain as a possible source of transmission to the community [47].

A multicentric study of food products from five European countries reported that one in every two food samples tested was shown to contain a member of the KpSC, and was mainly found in chicken meat and ready-to-eat salads. Kp1 isolates predominated (90.8%) and were found in the tested chicken and salad samples. Kp3 was next highest (6.1%) and shown to be at a much higher rate in salads (18%) than chicken (1%). Similarly, Kp2 (2.3%) was identified in salads alone and Kp4 (0.8%) was found in chicken alone. Identical genotypes found in different food types and in different countries suggested that the potential exists for a high level of transmission across the food chain that could result in human colonisation [48].

It is evident that *Klebsiella* can be cultured from diverse ecologic niches and that animal and environmental sources can act as a reservoir for the bacterium, where *Klebsiella* transmission to humans through a combination of direct animal contact or food consumption is currently under-investigated.

**ADAPTIVE FEATURES OF KLEBSIELLA ACCOUNT FOR ITS REPERTOIRE OF RESISTANCE GENOTYPES AND VIRULENCE FEATURES**

*Klebsiella* species are known to have an open pangenome that facilitates the acquisition of exogenous DNA, such as various mobile genetic elements (MGEs). This feature enables the bacterium to adapt to the various niches in which it is found. For example, the accessory genome of
Klebsiella species aids in the adaptation of the bacterium to ecologic niches by mechanisms that include evasion of human host defence mechanisms. Similarly, AMR and virulence genes are disseminated via MGEs between loci on the genome (insertion sequences, integrons, and transposons) or between bacteria (integrative conjugative elements [ICEs] and plasmids), thus supporting bacterial survival when placed under selection [49]. As a consequence, MDR-hv Klebsiella species resistant to carbapenem-compounds, tigecyclines, and quinolones have emerged. MDR-Kp challenge the ability of clinicians and veterinarians alike to treat these infections.

Important K. pneumoniae virulence factors include the capsule (whose production is regulated by the capsule regulatory ram-encoding genes) and siderophores (that function to enable iron acquisition: through expression of enterobactin, yersiniabactin, salmochelin and aerobactin; iron transport: ABC-transporter Kfu; and iron suppression: Fur). Lipopolysaccharide [LPS], O-antigens that avoid complement-mediated killing, synthesis is also of importance, as are adhesins, including the type 3 fimbriae for biofilm formation, outer membrane proteins [e.g., ompK genes to protect against neutrophil phagocytosis], type 6 secretion systems [e.g., phospholipase D family protein (PLD1) and one of the type 6 lipase effectors (Tle1), which cause destruction by injecting proteins into target cells] [50]. These features give rise to hv-Kp, a pathotype reported to contribute to increased morbidity and mortality.

In contrast, virulence factors of the KoSC have been less well-studied. Some 14 capsular polysaccharide K-antigens have been identified, with 12 K types being recognised in the KoSC clade and shown to be present in K. pneumoniae (K157 and K164 were identified only in the KoSC). While no LPS O-antigens have been published for the KoSC, seven O types have been identified, all of which have been reported in K. pneumoniae. The only known virulence factor for the KoSC is the AAHC–associated cytotoxin production of tilimycin and tilivalline [51]; however, there are some shared virulence factor–encoding genes with K. pneumoniae; including matB, which codes for capsule production, cf29a, fimA, fimH, mkABCDF, and pilQ, which code for biofilm formation, kfuBC, which codes for iron uptake, and ureA, which codes for urease activity [35].

Biofilm formation is thought to be involved in 65%–80% of bacterial infections reported in the developed world [52,53]. An important virulence feature expressed by K. pneumoniae is its ability to form a thick biofilm on living and abiotic surfaces, iron metabolism (siderophore down-regulation to trigger colonisation and infection, as well as immune system evasion), quorum sensing [QS] (types 1 and 2 QS for intra- and inter-species communication for coordinated behaviour of bacterial communities in a biofilm), and its ability to form collaborative biofilms with other bacterial species, such as Pseudomonas aeruginosa and P. protegens [55]. Indeed, vaccines to target these virulence factors have been developed and are in clinical trials [56]. Type III fimbriae have also been detected in K. oxytoca [57], and in AAHC 70% of K. oxytoca isolates are moderate biofilm formers [58].

MDR- and hv-Kp are usually thought of as distinct pathotypes of K. pneumoniae. Based on the comments above, MDR and hv-Kp can be distinguished based on the nature of their genomes. Originally, MDR and hv-Kp were thought to be non–overlapping, but are now being increasingly recognised and converging, thus giving rise to MDR-hv-Kp strains. Bacterial adaptation to any ecologic niche requires the acquisition of genes that confer a selective advantage. Given the open pangenome nature of this bacterium, AMR- and virulence–encoding genes can be acquired and disseminated between Klebsiella to enable this process.

**ONE HEALTH AND THE RELEVANCE OF KLEBSIELLA SPECIES**

Considering Klebsiella and its ubiquitous distribution across the three domains of One Health, it is not surprising to find that these bacteria are being identified while also being shown to be increasingly resistant to a repertoire of antibiotics (Fig 2). As highlighted by this review, antibiotic selection pressure, interspecies dissemination, and clonal expansion among the Klebsiella strains have been observed, which contributes to the successful spread of Klebsiella-AMR. Antimicrobial agents are used to treat disease in China, but these compounds can also be used to promote growth in large-scale poultry and pig farm production units, with lesser volumes being used in cow and sheep farms. The selective pressure that this imposes reflects the rate of AMR, as reported in one study of 189 K. pneumoniae collected from these 4 species of food-producing animals, in addition to human hospital samples. The rate of MDR–expressing bacteria was shown to be highest (93.6%) among pig isolates, followed by humans (90.4%), chickens (88.9%), cows (52%), and sheep (50%). The susceptibility of these bacteria cultured from these 5 mammalian hosts were tested against a panel of 15 antimicrobial compounds. The data obtained showed that meropenem had the lowest level of resistance recorded (11.6% of isolates were resistant) and ciprofloxacin had the highest level of resistance (77.8% of isolates were resistant). The nosocomial isolates elaborated higher resistance levels to all 15 antimicrobial compounds. Similarly, all 5 animal hosts showed resistance rates > 50% to ciprofloxacin and tetracycline. The prevalence of ciprofloxacin-resistant
isolates was higher in chickens (82.2%) and pigs (87.2%), considering fluoroquinolones are widely used in food-producing animals. In contrast, among animal-associated *K. pneumoniae*, most were susceptible to gatifloxacin, imipenem, and meropenem compounds that are not indicated for use in food-producing animals. Further evidence for the transmission of *K. pneumoniae* between humans and animals involved the occurrence of ST in animals being detected in hospital settings in China; ST11 was identified in all 5 of the hosts. In addition, ST235 and ST258 were in high abundance in humans, pigs, and chickens [59].

Colistin, otherwise known as polymyxin E, is an anti-biotic that has been clinically reintroduced as a last-resort agent due to the rise in MDR-expressing bacteria. Mobile colistin resistance (*mcr*) gene acquisition has been reported to be rapidly disseminated by horizontal gene transfer (HGT) among the *Enterobacteriaceae*. A broad study from a *One Health* perspective was conducted in which 2855 clinical *Klebsiella* genomes were screened for all 10 *mcr* homologs across 5 years in a Chinese hospital (2013-2018). Twenty (0.7%) of these genomes were positive for *mcr*, of which there were 6 variants (*mcr*-1.1, *mcr*-8.1, *mcr*-8.2, *mcr*-9.1, *mcr*-9.2, and *mcr*-10.1). Both *mcr*-9- and *mcr*-10- were susceptible to colistin and *mcr*-10.1 was discovered in *K. pneumoniae*, *K. quasipneumoniae* subsp. *quasipneumoniae*, and *K. variicola*. The remaining variants were all found solely in *K. pneumoniae*. Plasmid typing showed that IncFIκ, IncHI2, IncI2, and IncX4 (in order) were the most common incompatibility groups identified among these variants. Analysis of the *mcr*-carrying plasmids in GenBank noted highly comparable (> 75% coverage and > 98.5% nucleotide identity) plasmid backbones among the genomes of isolates cultured in chickens, pigs, silver gulls, hospital sewage, and wastewater treatment plants. Because colistin has been more readily used in animal industry than in human healthcare settings, identification of similar *mcr* variants in humans, animals, and the environment suggests that colistin resistance transmission can be observed in a *One Health* context. These plasmids also share additional genetic markers, such as other AMR-encoding genes conferring resistance to several classes of antibiotics including those not clinically-associated, a finding that suggests that the occurrence of *mcr* in these *Klebsiella* isolates may be due to waste-sources and food-producing animals and not due to clinical colistin selective pressure [60]. Evidence of inter-species and -patient transmission events was observed in a hospital in The Netherlands. In this study 21 isolates from 14 patients were shown to be positive for *mcr*-1-containing plasmids, including IncX4, IncI2(delta), IncHI2, IncHI2/IncN, and IncHI2/IncQ. These plasmids were found in *K. pneumoniae*, *Escherichia coli*, and *Kluyvera georgiana*, and on the chromosome of *K. pneumoniae* ST147 [an ST of international high-risk] [61]. These *Klebsiella*-associated plasmid transmission events carrying the broadest AMR gene repertoires amongst humans, animals, and the environment is an evolutionary process in need of urgent remedial action.

There is a compelling case to be made to monitor *Klebsiella* in all axes of *One Health*, including healthcare settings, animals (companion animals, food-producing animals, and exotics), and in the broader environment (e.g. plants and drinking water). To facilitate the initial steps in this process, some useful assembly-based tools for genomic surveillance of *K. pneumoniae* are already available, including Pathogenwatch, Kaptive, and Kleborate. Pathogenwatch (https://pathogen.watch/) is an online platform that implements genomic analytics for bacterial genera, including *Klebsiella*. These analytics, including phylogenetics, multilocus sequence typing (MLST), core genome MLST (cgMLST), AMR, virulence gene calling, replicon typing, and identifying loci encoding O- and K-antigens. These features are integrated with

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**FIGURE 2** | A conceptual schematic describing *One Health* interconnections that bacteria can harness to disseminate AMR. This was created in Biorender.com.
epidemiologic data, and visualization tools provided to aid in global surveillance. Features of Pathogenwatch include the BIGSdb software (http://bigdb.pasteur.fr/klebsiella/), which can be used to perform MLST and cgMLST, Kleborate (http://github.com/katholt/Kleborate) which is used to identify AMR and virulence loci, O- and K-loci, and wzi genes (the BIGSdb platform screens the genomes against a wzi database to predict K-type), and Haptive (https://github.com/katholt/kaptive) which performs a more robust capsule and LPS locus typing algorithm [62-65]. Not only will these technologies help elucidate the epidemiology of Klebsiella, but the technologies may also help identify targets for novel control strategies, such as vaccines.

CONCLUSIONS

Our environment has all too often been a neglected component part of One Health [66]. Consequently, when Klebsiella is considered, it is not surprising to find this opportunistic pathogen in the various constituent domains, a feature that consequently may explain why Klebsiella should be regarded as an under-appreciated zoonotic hazard [67]. The recent taxonomic revisions of the KpSC and KoSC clades, together with the lag in development and implementation of accurate diagnostic protocols for this genus, has contributed to this fact. Nonetheless, the extensive antibiotic resistance and hypervirulence phenotypes that can be expressed among isolates of importance to One Health need to be better understood. This feature warrants a reconsideration of how surveillance for this bacterium could be improved, given the evidence presented in this short review.

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CONFLICTS OF INTEREST

None to declare.

REFERENCES


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In 2002 Professor Fanning was appointed to the Chair of Food Safety and Zoonoses at University College Dublin and is the Director of the UCD Centre for Food Safety. Current research themes of the centre include studies related to antimicrobial resistance and bacterial transmission across the food chain, and these are focused at extending our understanding of those mechanisms that enable bacteria to adapt to food production environments. This research work is underpinned using molecular methods, including next-generation sequencing (NGS), among others. Professor Fanning’s research group has an interest in *Salmonella* and *Cronobacter*, related to low-moisture conditions and associated food matrices, such as powdered infant formula (PIF).

Professor Fanning has published more than 370 original experimental papers, along with 32 review papers, 28 book chapters and 2 text books. He is a member of the editorial boards of international journals, including *Foodborne Pathogens and Disease*, *Journal of Food Protection*, *Microbial Drug Resistance*, and *One Health Advances*, and is an editor of a further two journals *Research in Microbiology* and *FEMS Microbiology Letters*.

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