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Multi-drug resistant *Klebsiella pneumoniae* in foods of animal origin: A review

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Abstract

Klebsiella pneumoniae poses a significant threat to public health, as it is a leading cause of multidrug-resistant infections in hospitalized patients. This opportunistic pathogen is known to transfer antimicrobial resistance genes to other bacteria, exacerbating the issue of antibiotic resistance. Despite extensive research on *K. pneumoniae*, its ecology, pathogenicity, and population structure remain elusive. However, one aspect that has been overlooked is its ability to cause food-borne infections. While the reports on food-borne *K. pneumoniae* are limited, its potential to cause severe infections such as liver abscesses, septicaemia, and diarrhea in humans, as well as mastitis, urinary tract infections, and respiratory infections in animals, cannot be underestimated. The presence of *Klebsiella pneumoniae* in food is a concern because contaminated food can be a potential source of infection for humans. Foodborne transmission of *Klebsiella pneumoniae* can occur through the consumption of contaminated foods, such as raw vegetables, meat, and dairy products. The presence of multidrug-resistant strains of *K. pneumoniae* in the food chain can contribute to the spread of antimicrobial resistance, particularly among clinically relevant bacteria. In fact, beta lactamase and carbapenemase producing Enterobacteriaceae, including *K. pneumoniae*, have been classified as critical priority pathogens by the World Health Organization, highlighting the urgent need for new antimicrobial treatments. To combat AMR and *K. pneumoniae* infections, a comprehensive one health approach is necessary. Experts from medical, veterinary, pharmacology, microbiology, and the environment should work together to develop new strategies for preventing and treating infections. The development of novel antibiotics, antibiotic stewardship programs, and monitoring and surveillance efforts are also essential components of this approach.

Keywords: *Klebsiella pneumoniae*, Antimicrobial resistance, Food-borne infections, One Health approach, Novel antibiotics

Introduction

One of the most significant challenges in managing infectious diseases in humans and animals is the emergence of multi-drug-resistant Enterobacteriaceae, with *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella spp* being the most crucial. *K. pneumoniae* colonizes the skin and mucosa, causing UTIs, septicaemia, and pneumonia, and is a major nosocomial pathogen that spreads rapidly and can cause community-onset infections in both humans and animals. It is the second most prevalent Enterobacteriaceae species responsible for UTIs in humans and animals [35]. Extended spectrum beta-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae are typically multidrug-resistant, leading to significant complications in treatment. ESBL/carbapenemase-producing *K. pneumoniae* strains are often reported worldwide, and their spread is critical [10]. *K. pneumoniae* is the most common Klebsiella species that infects animals and causes mastitis, with losses due to Klebsiella mastitis being substantially greater than those due to *E. coli* mastitis [31]. The concern is that bacteria like *K. pneumoniae* in farm, food, and household environments may serve as reservoirs for the transfer of antibiotic resistance genes to pathogenic bacteria [39]. *K. pneumoniae* colonizes livestock, is found in retail meats and vegetables, and causes extraintestinal infections in humans [41]. The presence of *Klebsiella pneumoniae* in food is a concern because contaminated food can be a potential source of infection for humans. Foodborne transmission of *Klebsiella pneumoniae* can occur through the consumption of contaminated foods, such as raw vegetables, meat, and dairy products. In addition, food processing and handling practices can also contribute to the spread of the bacteria.

Foodborne diseases caused by pathogenic microorganisms pose a severe hazard to global public health. Until recently, most research on foodborne bacteria focused on common pathogens such as *Salmonella*, *Campylobacter*, *E. coli*, *Shigella*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* [33]. *K. pneumoniae* is a hospital-acquired and an important foodborne pathogen that can cause septicaemia, liver abscesses, and diarrhea in humans. While *K. pneumoniae* is not commonly considered a foodborne pathogen, antimicrobial-resistant strains have been isolated from various food sources worldwide, including vegetables, fish, chicken, beef, eggs, milk, and milk products [36]. Foodborne *K. pneumoniae* can cause nosocomial outbreaks. The presence of multidrug-resistant (MDR) *K. pneumoniae* strains in the food chain and their potential contribution to the resistome, particularly resistance of clinically relevant bacteria, should not be underestimated [3]. The World Health Organization (WHO) has identified food as one of the potential vehicles for transmission of antimicrobial-resistant bacteria to humans in its global action plan against antimicrobial resistance. The antimicrobial resistance (AMR) patterns of *Klebsiella pneumoniae* found in food varies depending on several factors, including the location and type of food samples tested, as well as the prevalence of antibiotic use in agriculture and veterinary medicine in that particular region. The WHO has classified carbapenem-resistant and third-generation cephalosporin-resistant Enterobacteriaceae, including *K. pneumoniae*, as critical priority pathogens in its list of antibiotic-resistant bacteria in need of novel treatments [40]. *Klebsiella pneumoniae* is among the group of seven bacterial species classified as ESKAPEE pathogens, which have been identified by the Centers for Disease Control and Prevention (CDC) as highly concerning drug-resistant organisms with significant implications for public health. Information, such as prevalence rates in retail food, antibiotic resistance profiles, and virulence characteristics, is needed to assess the risks of foodborne *K. pneumoniae* to public health. However, such knowledge gaps still exist, and therefore, this review was undertaken.

Klebsiella pneumoniae

Klebsiella pneumoniae, a Gram-negative bacterium, has been detected in various food sources, thus representing a significant public health concern. Of particular note is its pattern of antimicrobial resistance, which distinguishes it from other foodborne pathogens. Indeed, *Klebsiella pneumoniae* strains have been identified in raw, cooked, and processed meats, as well as vegetables and milk products, all of which may serve as potential sources of infection for humans. Moreover, the emergence of carbapenem-resistant strains of *Klebsiella pneumoniae* in food sources represents a particularly alarming development, given the limited treatment options available for such infections. As such, there is a pressing need for greater awareness and surveillance of this pathogen in food systems, and for the development of effective strategies to mitigate its impact on public health.

***K. pneumoniae* in Meat**

K. pneumoniae has been found in many meat sources worldwide, especially in beef. However, it is also present in other meats like pork, chicken, mutton, and chevon. Studies from different countries have reported *K. pneumoniae* prevalence in chicken ranging from 3.6% to 60%. Out of 70 samples of beef, turkey, and chicken tested from grocery

stores, 31 were positive for *K. pneumoniae*. Moreover, all isolates of *K. pneumoniae* were resistant to certain antibiotics like ampicillin, tetracycline, streptomycin, gentamicin, and kanamycin. The most commonly detected genes responsible for antimicrobial resistance were *aadA1*, *tetA*, *blaSHV-1*, and *blaTEM-1*. (24). In a study conducted in Turkey, 21 *K. pneumoniae* isolates were recovered from 60 samples of raw meat and chicken. Among the *K. pneumoniae* isolates, 28.3% were found to be ESBL-producers, indicating that they are resistant to extended-spectrum beta-lactam antibiotics. Additionally, 55%, 53%, and 47% of the *K. pneumoniae* isolates exhibited virulence properties, such as siderophore production, serum resistance, and haemolytic activity, respectively (Gundogan *et al.*, 2010) [18]. In USA, a study on *K. pneumoniae* from both food and clinical samples was conducted in 2011-12. Thirty-two percent of the isolates from meat had resistance to multiple drugs, such as ampicillin, gentamicin, and tetracycline; this percentage was higher than that observed for the clinical samples [11]. A study on imported and locally available poultry meat in Ghana found 81 ESBL producing isolates out of 200 samples tested. Of the 81 isolates 35 were *K. pneumoniae*. *bla_{CTX-M-15}* gene was detected in 86% of the *K. pneumoniae* isolates. The most common sequence types detected were ST2570, ST147 and ST15 [14]. A multi-centric study in Europe reported that 60% of chicken samples tested harboured *K. pneumoniae* [34]. ESBL producing *K. pneumoniae* was isolated in 3.6% of the raw chicken meat samples tested in Turkiye [36]. In China, *K. pneumoniae* was found in fish, shrimp, raw chicken meat, frozen goods, and cooked foods (meat, vegetables, flour, and rice products). Two isolates tested positive for extended-spectrum beta-lactamase (ESBLs), while 19 were multidrug resistant (MDR). The isolates obtained from raw chicken had the highest resistance rate [19]. Ten isolates of *K. pneumoniae* isolated from raw milk and cow faeces in the same country tested positive for the *bla_{NDM-5}* gene [23]. One *mcr-8* carrying isolate of *K. pneumoniae* was recovered from Pig samples in China. The isolate contained multiple antimicrobial resistance genes, including the β -lactam resistance genes *bla_{SHV-1}* and *bla_{CTX-M-14}*, aminoglycoside resistance genes *strA*, *strB*, *armA*, and *aph(4)-Ia*, macrolide resistance genes *mph(E)* and *msr(E)*, quinolone resistance genes *oqxA* and *qnrB4*, sulfonamide resistance genes *sul1*, *sul2*, and *sul3*, tetracycline resistance genes *tet(A)*, *tet(B)*, and *tet* [34], and trimethoprim resistance gene *dfrA12* [38]. Nearly 82% of the meat tested (pork, chicken and beef) were positive for *Klebsiella pneumoniae* as per a study conducted in Greece [37]. A study conducted by Montso and coworkers in South Africa reported 32% prevalence of *Klebsiella pneumoniae* from retail beef samples [29]. Klaf and coworkers found the prevalence of *Klebsiella pneumoniae* to be 76% in retail beef samples in Iraq [25]. A study from Assam conducted by Das and coworkers reported that *K. pneumoniae* was isolated from 21.43% of beef samples [8]. *K. pneumoniae* was reported in 57% of the chevon samples tested in Anand, Gujarat [32]. Junaid *et al.* (2022) reported 36.4% (4/11) prevalence for *K. pneumoniae* in beef samples in Pakistan. A study from Iraq reported 56% (28/50) prevalence of *Klebsiella pneumoniae* from retail beef samples [1]. A study conducted in Indonesia found that 7.8% chicken cloacal swabs were positive for *K. pneumoniae*, whereas a similar study from China reported prevalence of 28% [20, 43]. Modh and coworkers investigated the prevalence of *K. pneumoniae* in chicken meat in Gujarat, India and found that nearly 6% of the samples tested were harboring the organism [28].

***K. pneumoniae* in Street foods**

A study on the presence of *K. pneumoniae* in retail foods was conducted in China and which tested many retail food samples meat balls, raw meat from different livestock species. They reported a high percentage of virulence genes in the *K. pneumoniae* isolates. The commonly reported virulent genes were *fimH*, *ureA*, *wabG*, and *uge*. High resistance was noticed in ampicillin followed by streptomycin, piperacillin and tetracycline. Nearly 18% isolates were multidrug resistant [44]. A similar study conducted in retail food samples in India revealed high percentage of paani puris and chaats were positive (28.12%) for *K. pneumoniae*. The study also showed the *K. pneumoniae* (27.12%) was the common contaminant in retail foods than *Escherichia coli* (22.88%). The isolates showed high resistance to ampicillin followed by cefipime, cefuroxime and cefotaxime. Eight isolates of *K. pneumoniae* were metallo beta lactamase producers and 2 isolates were positive for all the three ESBL genes like *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{TEM} [17]. A report from Shijiazhuang city, China states that 9.9% of the 998 food samples tested were detected with *Klebsiella pneumoniae*. A report from Benin, states that nearly 20% of the 216 street food samples tested in a study were positive for *K. pneumoniae* with 27% isolates resistant to imipenem and 20% isolates carrying ESBL genes [30].

***K. pneumoniae* in Milk and Milk products**

ESBL producing *K. pneumoniae* has been isolated from raw milk, milk products like khoa, ice cream, kareish and domiati cheese in various studies conducted in India [5], Indonesia [6] and Egypt [15]. A study conducted in West Bengal and Himachal Pradesh, India reported that nearly 3% and 27% of the bovine milk samples tested were positive for ESBL-producing *K. pneumoniae* respectively [4, 8]. Koovapra and coworkers conducted a multi-state study on the prevalence of ESBL-producing *K. pneumoniae* in India and found that 4.4%, 13% and 6% of bovine milk samples from West Bengal, Jharkhand and Mizoram carried ESBL-producing *K. pneumoniae* [26]. A study conducted in Lebanon found that nearly 23.4% of the milk samples from healthy cattle were positive for *K. pneumoniae* through MALDI-TOF-MS. All the *K. pneumoniae* were ESBL-producers [12].

***K. pneumoniae* in Fruits and Vegetables**

A South Korean study reported that 8.5% of the ready to eat vegetables were positive for the presence of *Klebsiella pneumoniae*. Of the 16 isolates, *bla*_{TEM-1} was detected in 69% of the samples, *bla*_{SHV} in 94% samples, *bla*_{CTX-M-14} in 50% samples and *bla*_{CTX-M-15} in 19% of the samples [22]. A study from Oman reported that out of 105 samples of fresh raw vegetables and fruits tested, 13 isolates of *K. pneumoniae* were recovered [2]. Mesbah Zekar and coworkers isolated 13 third generation cephalosporin resistant *K. pneumoniae* isolates from fresh fruits and vegetables in Algeria. 38.5% isolates harboured sulphonamide resistant genes (*sul1*, *sul2*) and 100% isolates harboured quinolone, beta lactam, aminoglycoside resistant genes [27]. *K. pneumoniae* is also established to be a contaminant in sugarcane juice which showed resistance to important antimicrobials such as gentamicin, kanamycin, and neomycin. Yang and coworkers in China isolated a mcr-1 carrying *K. pneumoniae* from fresh fruit samples. It also carried other resistant genes such as *bla*_{SHV-110}, *tetA*, *qnrS1* [42]. A study in Singapore found that 15% of RTE foods tested were detected with the presence of *K. pneumoniae* while 7% of them carried a virulence gene.

The same study found that 45% of raw food samples like vegetables, meat harboured the organism but none of them carried a virulence gene [20]. Diriba and coworkers reported that 12.3% of the food handlers had faecal carriage of *K. pneumoniae* in Ethiopia [13]. Ready to eat raw vegetables like carrot, tomatoes, pumpkins were established to contain *K. pneumoniae* with genes responsible for beta lactam, quinolone resistance through whole genome sequencing according to a study conducted in Europe [7]. *K. pneumoniae* was also isolated from cheese manufacturing plant and raw materials like milk powder and cheese cans [16]. A 2022 multicentric study report from Europe found alarming levels of *K. pneumoniae* in chicken meat (60%) and salads (30%) from 5 countries [34].

Conclusion

Klebsiella pneumoniae is a resilient bacteria that can survive in a variety of environments, including food processing and storage facilities. Once it is present in the food system, it can be difficult to eradicate completely. Therefore, it is important to take measures to prevent the spread of *Klebsiella* in the food system, including implementing good hygiene practices, ensuring proper cooking and storage temperatures. The emergence of drug-resistant *K. pneumoniae* infections is a major concern globally. This medically significant pathogen has the ability to infect various human tissues, including critical organs such as the brain, liver, circulation, lungs, and bladder. Despite being known for over a century, *K. pneumoniae* has not been thoroughly researched, and its virulence mechanisms remain unclear. Moreover, the emergence of *Klebsiella* clonal lineages that combine resistance and hypervirulence has become increasingly common in recent years. Addressing the issue of AMR and *K. pneumoniae* infections requires a comprehensive one health approach, involving collaboration and expertise from medical, veterinary, pharmacological, microbiological, and environmental sectors. Strategies such as developing novel antibiotics, promoting antibiotic stewardship, monitoring, and surveillance are critical in combating this issue.

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