



Original article

Detection of *Klebsiella pneumoniae* antibiotic-resistant genes: An impending source of multidrug resistance dissemination through raw food



Kashaf Junaid^{a,*}, Hasan Ejaz^a, Sonia Younas^b, Awadh Alanazi^a, Humaira Yasmeen^c, Abdul Rehman^d

^a Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Al Jouf 72388, Saudi Arabia

^b HKU-Pasteur Research Pole, School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China

^c Department of Microbiology and Molecular Genetics, The Women University Multan, Pakistan

^d Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan

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ABSTRACT

This study aimed to find out the prevalence and antimicrobial resistance profile of *Klebsiella pneumoniae* in raw food items. A total of 261 raw food items, including vegetables, fruits, meat, and milk samples, were collected and processed for isolation of *K. pneumoniae*. Further antimicrobial susceptibility testing and molecular analysis was done to analyze the drug resistance encoding genes. The prevalence rate of *K. pneumoniae* was found to be high (38%), and the raw milk samples were predominantly contaminated (19/51), followed by fruits (12/51), meat (11/51), and vegetables (9/51). However, no significant association was observed for the isolation of *K. pneumoniae* and any particular specimen. Among the isolates, 43% were extended-spectrum β -lactamase producers, 24% were AmpC, and 20% were carbapenemase producers. The highest rates of ESBLs and AmpC were observed in vegetables (cabbage, bell pepper, and spinach) and carbapenemases in raw chicken, fish, and raw meat samples. Notably, *bla*_{CTX-M} was the most prevalent, followed by *bla*_{SHV} and *bla*_{TEM}. Six *K. pneumoniae* possessed *bla*_{MOX}, and five possessed *bla*_{FOX} genes. Numerous carbapenemases were identified with a higher proportion of *bla*_{NDM}. This study indicates that raw vegetables, fruits, meat, and milk are exposed to contaminants. These findings imply a potential threat that drug-resistant *K. pneumoniae* pathogens could transmit to humans through raw vegetables, fruits, and meat.

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1. Introduction

Klebsiella pneumoniae is a widespread opportunistic bacterium that causes various human diseases. The bacteria are responsible for a number of serious diseases in humans and animals, including meningitis, bronchitis, bacteremia, pneumonia, and urinary infec-

tions (Siri et al., 2011). In addition to these infections, clinical management has become more challenging due to the emergence of multidrug resistance among *K. pneumoniae* strains. *K. pneumoniae* is linked with increased patient morbidity and mortality and is also a well-recognized hospital-acquired pathogen (Cabral et al., 2012).

The distribution of *K. pneumoniae* is confined to human and animal gastrointestinal and respiratory tracts and is present in various environmental sources, including domestic and wild animals (Wareth and Neubauer 2021). The bacteria are an opportunistic pathogen of humans and animals, and a common contaminant in many food items. Aside from the clinical setting, *K. pneumoniae* can also be found in meat, fresh vegetables, milk, fish, and street food and has been regarded as a significant foodborne pathogen (Haryani et al., 2007, Kim et al., 2015, Davis and Price 2016, Kakatkar et al., 2017). *K. pneumoniae* is included in category B in powdered infant formula, a hazard identification category provided by the guidelines on microorganisms by the United Nations

Abbreviations: ESBL, Extended spectrum beta lactamases; NCBI, National center for Biotechnology Information; CFU, Colony forming unit; MIC, Minimum inhibitory concentration.

* Corresponding author.

E-mail addresses: kjunaid@ju.edu.sa, kashaf_junaid@hotmail.com (K. Junaid).

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Food and Agriculture Organization and the World Health Organization (Meeting et al., 2004). Resistance to numerous routinely available drugs has manifested in public health problems because of an increased rate of extended-spectrum β -lactamases (ESBL) and plasmid-mediated AmpC β -lactamase enzyme-producing pathogens. These β -lactamase enzymes are usually reported in gram-negative bacteria, for instance, *Escherichia coli* and *K. pneumoniae*. These bacteria could impose a threat on consumers as they may disseminate during food production and processing, such as the slaughter process (Kurittu et al., 2021). Animals have been identified as the main reservoir for ESBL microorganisms, and foods may contribute to the spread of resistance to humans through the food chain. As a result, several developed countries have established One Health approach programs to monitor antimicrobial resistance from food products (Founou et al., 2016).

Antibiotic-resistant bacteria are disseminated through the food-producing animals through feces. Fecal animal waste containing resistant bacteria can move around farms, slaughterhouses, and meat processing units (Doosti et al., 2015). Antimicrobial use in animals is evident in the high prevalence of antimicrobial resistance in human populations. More antimicrobial compounds are utilized in food-producing animals as the need for animal source nourishment grows in conjunction with the human population, mostly in lower middle income countries. Despite the high risk of transmission to humans through consuming contaminated food, only a few studies have been done; hence, the available data is limited. This research aimed to assess the prevalence, antimicrobial susceptibilities, and gene encoding for β -lactamases isolated from raw food items.

2. Materials and methods

2.1. Study design

The study was conducted prospectively on food bacterial isolates. The study followed the World Medical Association's ethical guidelines and the Declaration of Helsinki in handling bacterial strains to protect the population. Only *K. pneumoniae* strains were included in the study, and the remaining bacterial strains were excluded.

2.2. Sample collection

A total of 261 raw food samples were collected from different districts of Punjab, Pakistan. All specimens were obtained from roadside vendors, local markets, and supermarkets, including 101 roadside vendor samples, 89 from local markets, and 71 from the supermarkets. The food items included in this study are listed in Table 1. The food samples were collected aseptically in gamma-sterilized plastic pouches, whereas liquid food samples were taken in sterile screw-capped glass bottles. The specimens were stored at 4 °C until further processing.

2.3. Isolation of *K. pneumoniae* from food samples

All the food samples (25 g for solid and 10 ml for liquid) were suspended in 90 ml enrichment broth and homogenized for 30 s at 230 rpm. The suspended samples were incubated at 37 °C for 24 h before being cultured over MacConkey agar and incubated for another 24 h at 37 °C. Lactose fermenting colonies exhibiting a pink mucoid appearance were selected for further biochemical identification and API 20E confirmation. The genomic DNA of the phenotypically characterized *K. pneumoniae* strains was extracted as previously described (Boye and Hansen 2003). PCR was used to establish that the isolates were *K. pneumoniae* using the primer

sequence already reported; as quality control, ATCC *K. pneumoniae* (13883) was used. PCR conditions were followed as initial denaturation at 95 °C for 4 min was followed by 30 cycles of 15 s at 95 °C, 55 °C for one minute, and 72 °C for one minute, followed by a 5-minute extension step at 72 °C.

2.3.1. Minimal inhibitory concentrations

Minimal inhibitory concentrations (MICs) were determined using the broth microdilution technique by testing in vitro antimicrobial sensitivity for recommended drugs against gram-negative bacteria. The isolated colonies were aseptically mixed well in saline and dissolved to get a homogenous mixture comparable to 0.5 McFarland standard and dispensed in the appropriate microdilution plate. Kirby–Bauer disk diffusion technique using Mueller Hinton (MH) agar was applied in the phenotypic characterization of certain bacterial enzymes. The antibiotic discs were placed on the MH agar media after bacterial inoculation and incubated at 37 °C for 18–24 h. MIC breakpoints and inhibition zones were interpreted as *K. pneumoniae* sensitive or resistant to each antibiotic (Institute 2021).

2.4. Phenotypic screening of ESBL, AmpC, and carbapenemases

Each isolate of *K. pneumoniae* was phenotypically categorized to determine the occurrence of enzymes responsible for antibiotic hydrolysis. ESBLs were characterized based on the keyhole mechanisms using a co-amoxiclav and one of the cephalosporins (cef-tazidime and cefotaxime) on the MH agar plate (Begum et al., 2013). The use of cefoxitin disc with and without the addition of boronic acid helped to identify the AmpC β -lactamase. The inhibition zone expanded with the addition of boronic acid, representing AmpC presence (Younas et al., 2018). Carbapenemase production was screened with the resistance to one of the carbapenem, and the confirmation was done by the modified Hodge test (Junaid 2021).

2.5. Molecular characterization of ESBLs, AmpC, and carbapenemase encoding genes

As previously reported, a polymerase chain reaction was performed to determine genes encoding ESBLs, AmpC, and carbapenemase, using specific primers for these genes (Guo et al., 2016, Ghanavati et al., 2017). The amplified genes were detected by agarose gel (1%) electrophoresis using 1 × TBE buffer, stained with gel loading dye (6 ×), and visualized with the transilluminator. The amplified products were submitted for gene sequencing, and FinchTV was used to analyze the data. Tools such as BlastN and BlastP (National Center for Biotechnology Information [NCBI]) were used to identify genetic variants.

2.6. Statistical analysis

Descriptive analysis with SPSS (V. 26.0) was used for each variable to determine the frequencies and percentages. The p-value was calculated using the Chi-square test.

3. Results

3.1. Contamination of raw food with *K. pneumoniae*

A total of 261 raw food samples were examined in this study from vegetables, n = 72; fruits, n = 56; meat, n = 74; and milk, n = 59. Of these samples, 147 (43.7%) food items exhibited bacterial growth, including 30 vegetables, 25 fruits, 34 milk samples, and 25 meat samples. The average colony-forming unit (CFU) count was

Table 1

Number of culture-positive raw food samples (n = 261).

Raw food categories		Total No	Culture positive No (%)	Culture negative No (%)
Vegetable (n = 72)	Spinach	12	5 (41.7%)	7 (58.3%)
	Ginger	9	3 (33.3%)	6 (66.7%)
	Bell Pepper	8	2 (25%)	6 (75%)
	Cucumber	4	1 (25%)	3 (75%)
	Carrots	6	2 (33.3%)	4 (66.7%)
	Radish	9	2 (22.2%)	7 (30%)
	Cabbage	10	7 (70%)	3 (30%)
	Parsley	8	5 (62.5%)	3 (37.5%)
	Lettuce	6	3 (50%)	3 (50%)
	Raw Milk from local shops	32	23 (71.9%)	9 (28.1%)
Milk (n = 59)	Raw milk from dairy farms	27	11 (40.7%)	16 (59.3%)
	Chicken	13	7 (53.8%)	6 (46.2%)
Meat (n = 74)	Beef Diced/Minced	11	4 (36.4%)	7 (63.6%)
	Mutton	16	6 (37.5%)	10 (62.5%)
	Fish	14	5 (35.7%)	9 (64.3%)
	Shrimp	11	2 (18.2%)	9 (81.8%)
	Prawns	9	1 (11.1%)	8 (88.9%)
Fruits (n = 56)	Grapes	19	10 (52.6%)	9 (47.4%)
	Pears	17	5 (29.4%)	12 (70.6%)
	Strawberries	13	7 (53.8%)	6 (46.2%)
	Peaches	7	3 (42.9%)	4 (57.1%)
Total		261	114 (43.7%)	147 (56.3%)

high for vegetable 6.5×10^4 followed by milk 5.7×10^4 meat, 4.9×10^3 , and milk samples 3.1×10^3 . The lowest CFU was recorded in fruit samples 2.6×10^2 . Among the vegetables, the highest rates of contamination were observed in parsley (62.5%) and lettuce (50%). Raw milk collected from the local market had a high prevalence of contaminants (71.9%). The chicken had the highest rate of contaminants (53.8%), then minced beef, mutton, and seafood items. For the fruits, the most culture-positive samples were taken from strawberries (53.8%), grapes (52.6%), and peaches (43.7%).

Fig. 1 shows the schematic isolation of 51 *K. pneumoniae* strains from 261 food specimens, which yielded several ESBL, AmpC, and carbapenemase-producing *K. pneumoniae*.

From the 114 culture-positive samples, 51 (38%) *K. pneumoniae* were isolated concurrently from 9 vegetable, 12 fruit, 11 meat, and 19 milk samples. The rest of the 83 (62%) isolates were other gram-negative bacterial strains. The highest rate of *K. pneumoniae* contamination was observed in raw milk samples, followed by fruits, meat, and vegetables. However, a significant relationship was not

found for the isolation of *K. pneumoniae* with any specific sample, as presented in Table 2.

Results showed that food items collected from the vendors had the highest contamination rate compared to local stores and the supermarkets. *K. pneumoniae* were isolated at a higher rate (45.1%) from the vendors' food items than local stores and the supermarkets, as shown in Fig. 2.

3.2. Antimicrobial resistance profile

The antimicrobial resistance profile of *K. pneumoniae* showed varied resistance patterns against different classes of antibiotics. The least resistance patterns were observed against colistin, cotrimoxazole, and tigecycline. The resistance profile for other classes of drugs is shown in Table 3. The MICs inhibit 50% growth (MIC_{50}), and 90% growth (MIC_{90}) of *K. pneumoniae* isolates were observed against each tested antibacterial drug (Table 3).

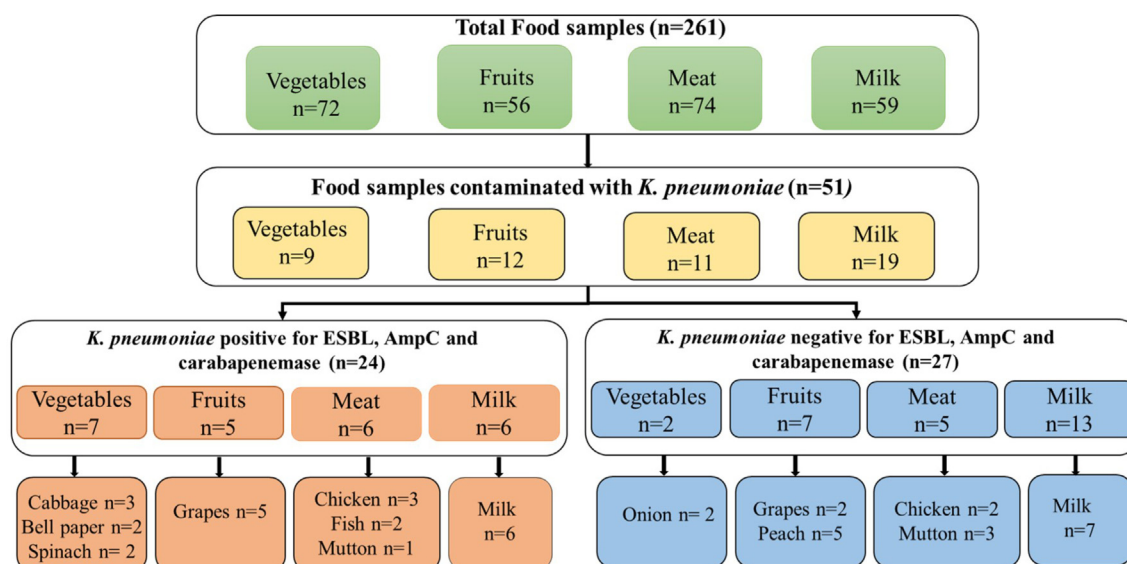
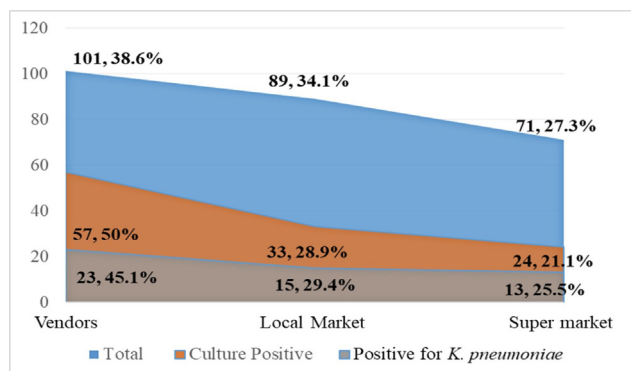
**Fig. 1.** Schematic isolation of 51 *K. pneumoniae* strains from 261 food specimens.

Table 2Frequency of *K. pneumoniae* strains from different food items (n = 51).

Food Categories	No of culture-positive samples (n = 114)	Positive for <i>K. pneumoniae</i> (n = 51)	Other gram-negative isolates (n = 83)	p-value
Vegetable	30	9 (17.7%)	32 (38.5%)	0.06
Fruits	25	12 (48%)	20 (24.1%)	0.12
Meat	25	11 (44%)	12 (14.5%)	0.09
Milk	34	19 (37.3%)	19 (22.9%)	0.39
Chi-square test used to calculate the p-value				

**Fig. 2.** Contamination rate of food samples from different sources (n = 261).

3.3. Detection of AmpC β -lactamases, ESBLs, and carbapenemase production

Of the 51 *K. pneumoniae* isolates from different food samples, 22 (43%) exhibited ESBL production, 12 (24%) AmpC, and 10 (20%) carbapenemase production. Of the 22 ESBLs, 31.8% were found in vegetables, 27.3% milk, 22.7% fruits, and 18.1% meat. Similarly, AmpC production was remarkably seen in vegetable samples [4/12 (18.1%)] than in other food samples. However, carbapenemase frequency was seen higher in meat samples [6/10 (27.2%)] compared to other food categories (Table 4).

3.4. Genotypic characterization of AmpC β -lactamases, ESBLs, and carbapenemase production

All 51 isolates of *K. pneumoniae* were screened by multiplex PCR analysis for ESBL gene variants. We detected a higher number of *bla*_{CTX-M}, followed by *bla*_{SHV} and *bla*_{TEM}. Six *K. pneumoniae* exhibited

*bla*_{MOX} and five *bla*_{FOX} genes. Several carbapenemases were detected in which a higher proportion of *bla*_{NDM} was observed. Table 5 shows numbers of different gene determinants in *K. pneumoniae* isolates.

4. Discussion

Antibiotic resistance is the most significant health challenge of the 21st century. Drug resistance is a global health concern, as it involves the transfer of bacteria and genes between humans, animals, and the environment. Antibiotic resistance and Infectious diseases expansion and dissemination are at an unprecedented rate worldwide, which forecast the global food demand is expected to rise substantially by 2100 (Jones et al., 2008). A significant rise in crop agricultural and livestock production will inflate agrarian usage of antibiotics, pesticides, fertilizer, and contact rates between humans and domestic and wild animals to fulfill the requirement of food for 11 billion people, all with consequences of newly emerging infectious agents. Vegetables and fruits can be contaminated during the farm-to-table cycle at the pre-harvest, harvest, and post-harvest phases.

Manure treatment, irrigation with polluted sewage water, agriculture land with a high population of pathogenic organisms, and other factors contribute to contamination of fruit and vegetable plants/trees during harvesting (Mathur et al., 2014). Consumption of contaminated food can cause food poisoning outbreaks. This situation draws our attention to assessing the antimicrobial resistance and genomic diversity in *K. pneumoniae* in raw food samples in Pakistan. For this purpose, 261 food samples of various categories were tested to determine the contamination from drug-resistant *K. pneumoniae*.

In this study, *K. pneumoniae* isolates were obtained from raw food items with an incidence of 38%, indicating that the contamination rate of *K. pneumoniae* is frequent in Pakistan. A similar study

Table 3Antibiotic resistance pattern of *K. pneumoniae* with MIC₅₀ and MIC₉₀ (n = 51).

Antibiotics	Breakpoint	MIC ₅₀	MIC ₉₀	% Resistance in isolates from food sources								Total Resistance	
				Vegetables n (%)		Fruits n (%)		Milk n (%)		Meat n (%)		Frequency	%
Aztreonam	≥ 16	64	64	6	27%	7	31%	5	22%	4	18%	22	44.00
Amikacin	≥ 64	6	64	2	12%	3	18%	4	25%	7	43%	16	32.00
Gentamicin	≥ 16	32	58	3	18%	4	24%	4	24%	6	36%	17	33.00
Cefuroxime	≥ 32	64	72	5	22%	6	26%	4	17%	8	35%	23	45.00
Cefoxitin	≥ 32	22	60	0	0%	1	9%	3	28%	7	65%	11	21.00
Ceftriaxone	≥ 4	12	32	2	11%	3	16%	5	26%	9	48%	19	37.00
Ceftazidime	≥ 16	32	63	4	19%	6	29%	6	29%	5	24%	21	41.00
Cefotaxime	≥ 4	12	64	4	20%	3	15%	4	20%	9	45%	20	39.00
Cefepime	≥ 16	12	64	2	18%	1	9%	1	9%	7	61%	11	22.37
Ciprofloxacin	≥ 1	8	64	1	9%	1	9%	2	18%	7	61%	11	22.37
Levofloxacin	≥ 2	8	32	2	16%	2	16%	2	16%	6	47%	13	25.00
Imipenem	≥ 4	32	64	0	0%	2	18%	3	27%	6	53%	11	22.00
Meropenem	≥ 4	32	64	1	9%	0	0%	4	37%	6	56%	11	21.00
Piperacillin-Tazobactam	≥ 128/4	8/4	128/4	1	12%	1	12%	1	12%	5	62%	8	15.79
Colistin	≥ 4	2	4	0	0%	0	0%	0	0%	1	98%	1	2.00
Co-trimoxazole	≥ 4	1	2	0	0%	0	0%	0	0%	1	100%	1	1.96
Tigecycline	≥ 2	1	2	0	0%	1	57%	0	0%	1	57%	2	3.45

Table 4Raw food samples exhibiting AmpC β -lactamases, extended-spectrum β -lactamases, and carbapenemase production.

Food Item	No. of samples	ESBL producers	AmpC producers	Carbapenemase
Fruits	12	5 (22.7%)	3 (25%)	2 (20%)
Vegetables	9	7 (31.8%)	4 (33.3%)	1 (10%)
Milk	19	6 (27.3%)	2 (16.7%)	1 (10%)
Meat	11	4 (18.2%)	3 (25%)	6 (60%)
Total	51	22	12	10

Table 5Genetic characterization of AmpC β -lactamases, ESBLs, and carbapenemase-producing *K. pneumoniae*.

Resistance genes	Gene variants	Number (%)
ESBL genes (n = 38)	<i>bla</i> _{CTX-M-1}	12 (31.6%)
	<i>bla</i> _{CTX-M-2}	6 (15.8%)
	<i>bla</i> _{CTX-M-9}	3 (7.9%)
	<i>bla</i> _{SHV-12}	7 (18.4%)
	<i>bla</i> _{SHV-11}	7 (18.4%)
	<i>bla</i> _{TEM-1}	1 (2.6%)
	<i>bla</i> _{TEM-4}	2 (5.3%)
AmpC genes (n = 11)	<i>bla</i> _{MOX}	6 (54.5%)
	<i>bla</i> _{FOX}	5 (45.5%)
Carbapenemases (n = 17)	<i>bla</i> _{NDM-1}	7 (41.2%)
	<i>bla</i> _{NDM-5}	3 (17.6%)
	<i>bla</i> _{IMP}	2 (11.8%)
	<i>bla</i> _{VIM}	3 (17.6%)
	<i>bla</i> _{OXA-48}	2 (11.8%)

in India reported the incidence of bacterial contamination and antimicrobial resistance of microorganisms in fresh fruits and vegetables (Saksena et al., 2020). Studies from South Asia have also reported the contamination rate and frequency of different microorganisms isolated from fresh fruits and vegetables, such as *Klebsiella* spp., *Enterobacter* spp., *E. coli*, *Citrobacter* spp., and *Pseudomonas* spp. as the predominant bacterial strains (Viswanathan and Kaur 2001, Nipa et al., 2011). In our study, among the vegetables, the highest contamination rates were observed in parsley (62.5%) and lettuce (50%).

Many studies have reported the isolation of *K. pneumoniae* from various vegetables, such as carrots, iceberg lettuce, cucumber, and tomato (Boehme et al., 2004, Shahid et al., 2009). A study from Spain reported multidrug-resistant *K. pneumoniae* strains isolated from fresh vegetables (Falomir et al., 2013). We found that raw milk collected from the local market had a high prevalence of contaminants (71.9%).

K. pneumoniae is a prevalent etiological agent to cause bovine mastitis in dairy animals and might be found in cow's milk that has clinical and subclinical infections (Bhatt et al., 2012). Microbiome investigations of milk samples from a cow revealed *Klebsiella* as a frequent part of the dynamic microbial community linked with bovine mastitis (Bhatt et al., 2012). According to another study, *K. pneumoniae* has been identified in lactating cows' feces and found in bulk tank milk (Ohnishi et al., 2013, Sudarwanto et al., 2015). Antibiotic resistance, notably ESBL-mediated resistance, is widespread among mastitis-associated *K. pneumoniae* infections.

A similar study was conducted in China to determine the prevalence of drug-resistant *K. pneumoniae* strains in raw and processed food samples (Guo et al., 2016). A 9.9% incidence rate was reported, which showed the presence of *K. pneumoniae* in food items in China. We also found that chicken had a higher rate of contamination (53.8%) than minced beef, mutton, and seafood items, which agrees with a previous study from China (Guo et al., 2016). Moreover, food items collected from vendors had a higher contamination rate of *K. pneumoniae* (45.1%) compared to local stores and the supermarkets.

The antimicrobial resistance profile of *K. pneumoniae* was varied, with the least resistance patterns observed against colistin, co-trimoxazole, and tigecycline, for which the findings are somewhat similar to a study from South Africa (Montso et al., 2019). We found 43% ESBL, 24% AmpC, and 20% carbapenemase production among the isolates. Of the 22 ESBLs, 31.8% were found in vegetables, 27.3% milk, 22.7% fruits, and 18.1% meat. *K. pneumoniae* recovered from retail meat frequently exhibits multidrug resistance, and ESBL-producing bacteria have also been found, mainly in retail chicken samples (Overdevest et al., 2014, Casella et al., 2015). Similarly, AmpC production was more significant in vegetable samples (4/12 [18.1%]) than in other food samples. However, carbapenemase frequency was higher in meat samples (6/10 [27.2%]) than other food categories. Upon characterization, we detected a higher number of *bla*_{CTX-M} was followed by *bla*_{SHV} and *bla*_{TEM}; this follows a report from China (Guo et al., 2016).

Six *K. pneumoniae* exhibited *bla*_{MOX} and five *bla*_{FOX} genes. Several carbapenemases were detected in which a higher proportion of *bla*_{NDM} was observed in our study, which raises a substantial concern. In animal husbandry, most of the antibiotics used for non-therapeutic objectives, for instance, to prevent infectious diseases and to enhance, are routinely discovered in cattle food at low and sublethal quantities, which reduce the growth rate of sensitive microbes (Woolhouse and Ward 2013, Zhu et al., 2017). Consequently, bacteria in livestock digestive tracts are under selective pressure to sustain antibiotic resistance genes (ARGs), increasing the relative abundance of resistant populations (Gullberg et al., 2011). Drug resistance is also becoming an eco problem when these ARGs spread to the surrounding environment, and ARGs are contaminants of rising concern (Rysz and Alvarez 2004). The widespread use of antibacterials as growth promoters in poultry and aquaculture sectors and the use of poultry litter as manure in agriculture could all be contributing factors to the dissemination of resistant genes in the environment and animal husbandry. The emergence and dissemination of multidrug-resistant bacteria pose a significant health and economic burden globally, demanding urgent attention. There is a need for antimicrobial stewardship and policies that can help break the chain of bacterial resistance. This study also has few limitations as the samples were collected from a small geographically localized region. High drug resistance microbes in the food sample were not explicitly related to the state. Moreover, it would be interesting to investigate the presence of virulence genes in foodborne isolates, which we could not do in this study. The presence of multidrug-resistant isolates and harboring virulent genes is a potential health risk.

5. Conclusion

This study discovered a greater rate of drug-resistant *K. pneumoniae* strains in food samples. The presence of drug resistance in raw food items (fruits, vegetables, and milk) indicates microbial resistance in the environment. The prevalence of different β -lactamases encoding genes such as CTX-M, TEM, SHV, and NDM highlights the urgent need for antimicrobial stewardship. Multidrug resistance in food *K. pneumoniae* strains jeopardizes the clinical utility of several antibiotic groups, which have shown elevated MIC₅₀. The situation could be severe if these strains develop resis-

tance to tigecycline, co-trimoxazole, colistin, and piperacillin-tazobactam, which are the only therapeutic substitutes in this study and presented with extremely low MIC₉₀. There is a dire need to restrict the irrational use of antibacterial drugs in humans, animal husbandry, and agriculture. Moreover, studies are urgently needed employing phylogenetic and whole-genome sequencing to target the drivers of antimicrobial drug resistance, virulence factors, and genetic diversity. These works will aid in understanding the population biology of pathogenic microbes isolated from non-clinical settings.

Consent for publication

All the authors consented to the paper publication.

Availability of data and material

The data used and analyzed during the current study available from the corresponding author.

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CRediT authorship contribution statement

Kashaf Junaid: Conceptualization, Data curation and Formal analysis, Funding acquisition, Final review and editing. **Hasan Ejaz:** Conceptualization, Data curation and Formal analysis, Funding acquisition, Final review and editing. **Sonia Younas:** Experiments and Validate the methods for this study, Write the original draft. **Awadh Alanazi:** Write the original draft. **Humaira Yasmeen:** Write the original draft, Experiments and Validate the methods for this study. **Abdul Rehman:** Conceptualization, Data curation and Formal analysis, Experiments and Validate the Methods for this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author's contribution

Kashaf Junaid, Hasan Ejaz, and Abdul Rehman designed the study and experiments, analyzed the data, applied statistical analysis. Sonia Younas and Humaira Yasmeen collected the samples and processed them. Awadh Alanazi helped in statistical analysis and drafting the manuscript. All authors critically evaluated the manuscript, and the final version was approved.

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