History of Medicine:Vol. 10 No. 2 (2024):1781-1800
DOI:https://doi.org/10.48047/HM.10.2.2024.1781-1800

A Study to evaluate the antimicrobial susceptibility pattern of isolated strains of Klebsiella pneumoniae

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ABSTRACT

Klebsiella pneumoniae is a deadly pathogen that can infect humans and livestock. *K. pneumoniae* causes an infection that is difficult to treat due to its ubiquitous presence and significant drug resistance. The present study was conducted to determine the prevalence of *Klebsiella pneumoniae* from raw bovine meat samples along with antibiotic resistance profiling of isolates. Isolation and identification of *Klebsiella pneumoniae* was done according to standard microbiological techniques and biochemical reactions respectively. The isolated strains were then subjected to the Kirby-Bauer disk diffusion method to detect antibiotic susceptibility. The results showed that out 100, 24(24%) were found positive for *K. pneumoniae* by biochemical and microscopic testing. The prevalence was higher in fresh beef (30%) samples than frozen samples (18%).The antimicrobial susceptibility test showed the maximum resistance was found against Ceftriaxone (54.17%), Ceftazidime (54.17%) and Cefotaxime (54.17%) while meropenem and imipenem showed maximum sensitivity for *K.pneumoniae.* The prevalence of multidrug-resistant isolates was 45.83%. Molecular detection of ESBL genes showed, the prevalence of $bla_{\text{CTX-M}}$36.36%, bla_{OXA} 27.27%, and bla_{TEM} 18.18% while none of the samples was detected positive for bla_{NDM} gene. This study indicates that the presence of *K. pneumoniae* is quite obvious in both fresh and frozen beef with multiple drug-resistance abilities. Carbapenem drugs are still the option to treat such food-borne infections.

Keywords: Klebsiella pneumonia, Prevalence, Molecular Detection, Antimicrobial Susceptibility, Food-Borne Infections

INTRODUCTION

The majority of microorganisms involved in food-borne illness are of animal origin(Busani et al., 2006). In developing world, up to one-third of the global population suffers food-borne diseases each

year. Over 2.2 million people die from diarrheal diseases that are food/water-borne each year (WHO, 2006). The fecal contamination of beef and chicken with members of *Enterobacteriaceae* family such as *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp. and Proteus spp. worries food safety specialists (Paterson, 2006). Effective frequency monitoring and accurate zoonotic pathogenic bacteria identification in food are essential for lowering the occurrence of food-borne illnesses and microbial contamination of food (Carroll et al., 2007).

Klebsiella species are abundant in nature and are found in water, soil and other surfaces(Martin & Bachman, 2018). In humans, *K. pneumoniae* often colonizes numerous mucosal surfaces, including gut and the upper respiratory tract, where colonization rates vary greatly depending on environment and exposure(Podschun et al., 2001). The WHO produced a list of infections for which new antibiotic development is urgently required in February 2017 to focus and direct research and development linked to new antibiotics. This lengthy list included the infections known as ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) (De Oliveira et al., 2020). ESKAPE pathogens have developed resistance mechanisms to oxazolidinones, lipopeptides, macrolides, fluoroquinolones, tetracyclines, β-lactams, β-lactam-β-lactamase inhibitor combinations, and antibiotics that are the last line of defence, including carbapenems, glycopeptides, and clinically unfavourable polymyxins, through genetic mutation and the acquisition. According to recent research, the frequency of *Klebsiell*a colonization ranges from 18.8 to 87.7% in Asia and 5 to 35% in Western nations(Marr & Russo, 2019).

Numerous virulence factors are present in *Klebsiella pneumoniae*. The pathogenesis of *K. pneumoniae* has historically been linked to four main elements: siderophores, adhesion factors and lipopolysaccharide. Capsular Polysaccharide was the first pathogenicity component of *Klebsiella* to be discovered (K-antigen). This antigen produces a thick hydrophilic capsule that gives *K. pneumoniae* colonies shiny and mucoid appearance on agar plates. Until now, K1, K2, and so-on have been assigned to at least 78 different K- antigen serotypes(Cao et al., 2022)

The ubiquitous *Klebsiella pneumoniae* species complex (KpSC), a major contributor to antibiotic-resistant human illnesses, is everywhere. Little is known about how food components affect pathogen colonization and transmission in people, despite the enormous risk that *K.pneumoniae* poses to public health. This is because there are no proven methods for determining if foods contain the *Klebsiella pneumoniae* species complex (KpSC). Numerous investigations have shown that KpSC members are abundant in chicken meat, supporting the idea that common people may become colonized with the *Klebsiella pneumoniae* species complex (KpSC) through food(Kislaya et al., 2022).

Numerous antibiotics, including B-lactams and aminoglycosides, are efficient in preventing and treating infections brought on by *K. pneumoniae*. The overuse and abuse of antibiotics, however, significantly lowers their efficiency and makes it harder to treat *K. pneumoniae*. This phenomenon is known as antimicrobial resistance (AMR)(Krause et al., 2016). The majority of antibiotic resistant *K. pneumoniae* isolates contain plasmids, which are the most significant carriers for antimicrobial resistance in MDR K. *pneumoniae(Navon-Venezia et al., 2017).*

K. pneumoniae is a colonizing important pathogen of animal and human that is a typical contaminant present in raw meat(Lee et al., 2004). *Klebsiella pneumoniae* causes illness in horses, cows, and companion animals(Ewers et al., 2014). *K. pneumoniae* is a significant pathogen that causes nosocomial infections, including UTIs, and it is commonly linked to resistance to the most important and high-priority antimicrobials. In humans, *K. pneumoniae* often colonizes the gut and occasionally causes GIT infections(Roca et al., 2015). Although recognized for its ability to cause pneumonia, *K. pneumoniae* also causes cystitis, osteomyelitis, meningitis, pyelonephritis, bacteremia, septicemia, wound infections, and liver abscess(Shon et al., 2015).Furthermore, rising antibiotic resistance among *Klebsiella pneumoniae* complicates the clinical treatment of severe infections(Muñoz et al., 2013).

The preferred treatment for treating severe infections brought on by extended-spectrum-lactamases produced by *Enterobacteriaceae* is carbapenems like imipenem and meropenem (ESBLs). In several hospital- and community-acquired *Enterobacteriaceae* rods, carbapenem resistance is now regularly seen. It is quite concerning because carbapenem-resistant *Enterobacteriaceae* (CRE) have been linked to treatment failure and significant mortality rates (Oho et al., 2021). Although several pathways for carbapenem resistance have been identified, the most prevalent one is the development of carbapenemases. Numerous clinically significant carbapenemases, such as the Guiana extended-spectrum metallo-β-lactamases (GES), *Klebsiella pneumoniae* carbapenemase (KPC), Verona integron-encoded metallo-β-lactamases (VIM), imipenem-hydrolyzing metallo-β-lactamases (IMP), Sao Paulo metallo-β-lactamase (SPM) (Zheng et al., 2013).

MATERIAL AND METHODS

Sample collection

A total of 100 fresh and frozen bovine meat samples were collected from different butcher shops and supermarkets by employing sterile techniques. Samples were then transported in an ice box within 2 hours to postgraduate research laboratory, Department of Microbiology, Government College University Faisalabad.

Isolation and identification of *Klebsiella pneumoniae*

25 grams of each meat sample was taken and properly chopped into smaller pieces. Peptone water was prepared and 10 ml of peptone water was taken in a test tube for each sample. Each sample was added to a separate peptone water-filled test tube and mixed thoroughly. Then all the test tubes were incubated aerobically for 24 hours at 37°C(Benie et al., 2017).

All the enriched samples were streaked on MacConkey's agar, a selective and differential media for isolating the non-fastidious Gram-negative rods, most dominantly the members of the family *Enterobacteriaceae*. The streaked plates were then incubated overnight at 37 °C aerobically. The pink-colored colonies with a sticky nature are the prominent features of *Klebsiella pneumoniae* on MacConkey's agar due to its lactose fermentation characteristic. The identification was done based on Gram's staining, morphological characters, cultural characters, and biochemical characteristics as described by the FDA Bacteriological Analytical Manual(Montso et al., 2019). Isolates were preserved in 10% glycerol preparation with tryptic soy broth and stored at -80°C till further study (Messele et al., 2017).

Gram staining

Gram staining was done to differentiate Gram-positive bacteria from Gram-negative bacteria. Observation of the slides was done under the microscope at 40X to focus and 100X to study the morphology of the isolates (Tripathi & Sapra, 2020).

Catalase test

This test was performed to distinguish catalase producers i.e. *Klebsiella pneumoniae* and non-catalase-producing organisms. When the catalase-producing microorganisms come in contact with hydrogen peroxide, it splits into water and oxygen. Oxygen bubbles can be seen in the catalase-producing bacteria (Nandi et al., 2019).

Oxidase test

It is a biochemical test for distinguishing *Klebsiella pneumoniae* because it does not secrete cytochrome C oxidase to contact with Tetramethyl-p-phenylenediamine, so no color changes occur and hence identified rapidly (Huang et al., 2020).

Indole test

This test is led to identify the organisms capable of tryptophanase production. When these tryptophanase producers are incubated in a medium containing tryptophan, they degrade it and convert it into a compound named, indole. This conversion is indicated when Kovac's reagent is added in medium and p-dimethylaminobenzaldehyde reacts with indole and produces a dye rosin dole (Nayak et al., 2020).

Methyl red test

This test is employed to identify those organisms, which have the ability to do mixed acid fermentation when enough carbohydrate is supplied to it. When glucose is provided in the medium, the organism converts it into lactic acid, formic acid, and succinic acid along with carbon dioxide and hydrogen. Methyl red is used as an indicator and when enough acid is produced in the medium and the pH lowers below 4.5, this indicator changes its color from yellow to red (Waghmode et al., 2020).

Voges-Proskauer test

This name was given to this test after two scientists who developed it in 1998. It is done to assess whether the organisms have the capability to ferment glucose and produce acetyl methyl carbinol from it. When Barrit's reagent A and B (40% KOH + alpha naphthol) is added to the MR-VP broth, the acetyl methyl carbinol is converted to diacetyl which condenses and gives a red color (Pisal & Yadav, 2021).

Molecular confirmation of *Klebsiella pneumoniae*

All the isolates of *Klebsiella pneumoniae* were subjected to a polymerase chain reaction for molecular confirmation. For this purpose, the genomic DNA of isolates were extracted with the help of a commercially available kit Gene JET Genomic DNA Purification Kit (Thermo Scientific, USA) as described by (Al-Agha et al., 2017).

Polymerase chain reaction (PCR)

For the confirmation of *Klebsiella pneumoniae* isolates, a Polymerase chain reaction was employed as defined by(El Aila et al., 2010). The mixture of 25µl reaction in PCR tubes was prepared which contained forward and reverse primers (1µl) each, (12µl) master mix, (DEPC) treated water (8µl), and (3µl) extracted DNA. The tube containing the mixture was placed in a thermal cycler for 35 cycles. The thermal cycler conditions were adjusted to 94°C for 05 min, 94°C for 30 sec/35 cycles, 55°C for 45 sec/35 cycles, 72°C for 45sec/35 cycles, and final extension at 72°C for10 min.

Table 1: List of primer sequences

Targeted gene	Primer details	Product size (bp)
KP_16S	F-5,- ATGTCGCAAGACCAGAGTGG-3’	657 (El Aila et al., 2010).
	R-5’-CACAACCTCCAAATCGACA	
Bla$_{TEM}$	F-5,- ATGAGTATTCAACATTTCCG -3’	862bp (Cho et al., 2014)
	R-5’- GACAGTTACCAATGCTTAATCA-3’	
bla$_{OXA}$	F- 5’-GCGTGGTTAAGGATGAACAC-3’	309bp (Liu et al., 2015)
	R-5’-CATCAAGTTCAACCCAACCG-3’	
Bla$_{NDM}$	F-5’-GGT TTG GCG ATC TGG TTT TC-3’	621bp (Candan et al., 2017)
	R-5’-CGG AAT GGC TCA CGA TC-3’	
Bla$_{CTXM}$	F-5’-CGTCACGCTGTTGTTAGGAA -3’	786bp (Bello-Lopez et al., 2017)
	R-5’-ACGGCTTTCTGCCTTAGGTT-3’	

Agarose Gel Electrophoresis

After PCR, the products of PCR were visualized through gel electrophoresis to check whether the target has amplified or not (Al-Agha et al., 2017).

Antibiotic susceptibility testing

After the confirmation of *Klebsiella pneumoniae* isolates by PCR, their antimicrobial susceptibility profiling was done using antibiotics as Gentamicin (10µg), amikacin (30µg), ampicillin(10µg), meropenem (10µg), ceftriaxone (30µg), amoxicillin (30µg), cefotaxime (30µg), ceftazidime (30µg), tetracycline (10µg), ciprofloxacin (5µg), imipenem (10µg) by Kirby-Bauer method(Gopalakrishnan et al., 2018).

Table 2: Antibiotic susceptibility pattern for *K. pneumoniae*

Antibiotic used	Resistance	Intermediate	Susceptible
Amoxicillin clavulanic acid (AMC)	≤13	14-17	≥18
Gentamicin (CN)	≤12	13-14	≥15
Ceftriaxone (CRO)	≤19	20-22	≥ 23
Ciprofloxacin (CIP)	≤ 21	22-25	≥ 26
Ceftazidime (CAZ)	≤ 17	18-20	≥ 21
Cefotaxime (CTX)	≤ 22	23-25	≥26
Amikacin (AK)	≥17	15-16	≥14
Imipenem (IPM)	≤19	20-22	≥23
Meropenem (MEM)	≤19	20-22	≥23
Tazobactam (TAZ)	≤20	21-24	≥25
Ampicillin (AMP)	≤ 13	14-16	≥17

McFarland Standard

In microbiology, the McFarland standards method is used for adjustment of the bacterial suspension turbidity before antibiotic susceptibility testing. The solutions of barium chloride (BaCl2.H2O) 1.175% and 1% sulphuric acid solution (H2SO4) were prepared for standardizing 0.5 McFarland standards. Both the solutions were mixed and they formed barium sulfate which caused the turbidity in the solution. This solution was stored in a falcon tube at room temperature (25°C). A spectrophotometer was used to check the optical density (OD) of the standard at 625 nm wavelength

and absorbance was also found in a considerable range of 0.08-0.1. The prepared 0.5 McFarland turbidity standard shows that cell density in prepared bacterial suspension is approximately $1.5x10^8$ CFU/ml (Ayati et al., 2022).

Table 3: Preparation of 0.5 McFarland Turbidity Standards

Reagents	Quantity (ml)
1.0% Sulphuric acid	9.95
1.18% Barium chloride dehydrate	0.05

Modified Kirby Bauer Disk Diffusion Technique

Mueller Hinton agar (Oxoid, UK) was prepared for antimicrobial susceptibility testing. The fresh bacterial suspension was compared with standards of 0.5 McFarland. The plates of agar were then swabbed with bacterial culture and antibiotic discs were implanted on the plates according to CLSI guidelines. Then incubation was given to the plates aerobically at 37˚C for 24 hrs. Zones of inhibition were measured after incubation and categorized isolates as resistant, sensitive, or intermediate (Khan et al., 2020).

RESULTS

Prevalence of *Klebsiella pneumoniae* in beef samples

In the current study, a total of 100 beef samples were collected and processed for isolation and identification of *Klebsiella pneumoniae*. The results indicated that 24 (24%) samples were positive for *K. pneumoniae*. The frequency in fresh beef samples was (15/50; 30%) in comparison to frozen beef samples (9/50; 18%) as shown in (Table 01 and Figures 01 a&b).

Table 01. Frequency of *Klebsiella pneumoniae* from fresh and frozen beef samples

Sample type	Total samples	Frequency of *K. pneumoniae*	Prevalence of *K. pneumonia*
Fresh beef	50	15	30%
Frozen beef	50	09	18%
Total	100	24	24%

(a) **(b)**

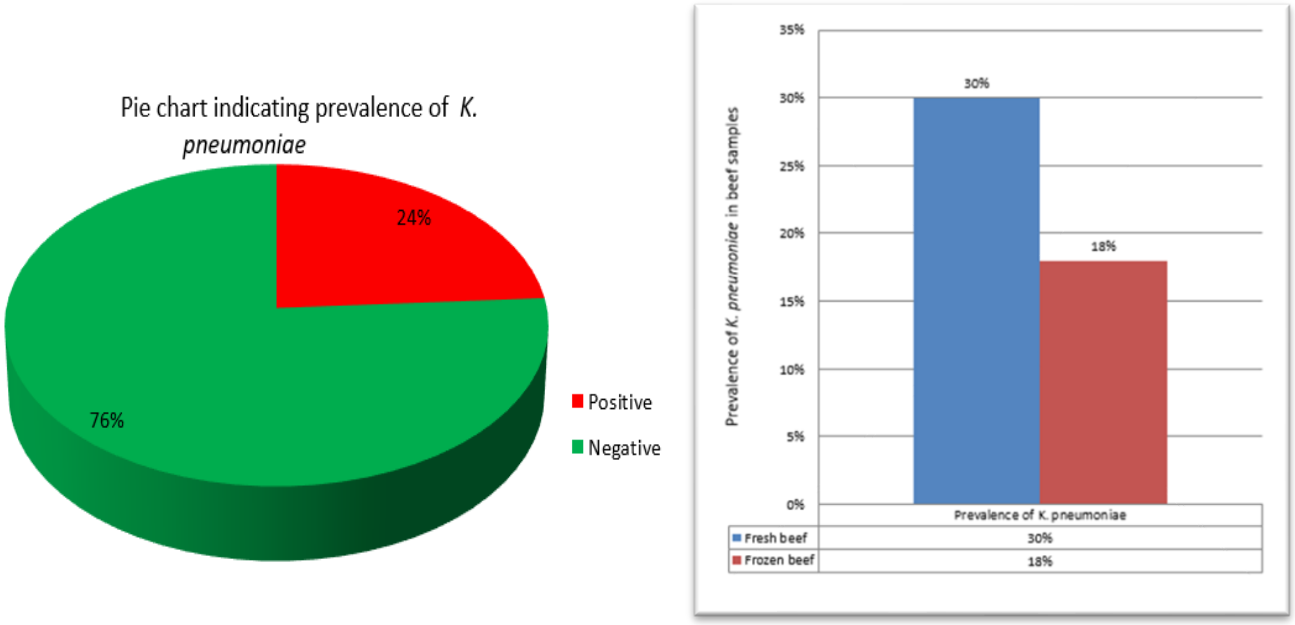

Figure 01: (a) Frequency of *Klebsiella pneumoniae* from fresh and frozen beef samples (b) Frequency of *K. pneumoniae* based on type of beef

Isolation and Identification

On MacConkey's agar, *Klebsiella pneumoniae* showed lactose fermenting light pink color colonies due to the production of acid as shown in (Figure 03). When Gram staining was applied to the isolates, very clear Gram-negative rods were observed under a microscope at 1000X magnification as shown in (Figure 04).

Figure 02: Mucoid pinkish sticky colonies of *K. pneumoniae* on MacConkey's agar

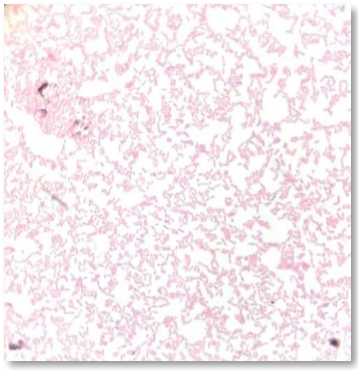

Figure 03: Pinkish colored Gram negative rods indicating *K. pneumoniae*

Biochemical profiling for *Klebsiella pneumoniae*

Different biochemical tests were performed for biochemical confirmation of *Klebsiella pneumonia* including oxidase, catalase, methyl red, Voges Proskauer and indole. For *K. pneumoniae* isolates, catalase and Voges Proskauer tests were positive while oxidase, methyl red and indole tests were negative. The results for biochemical profiling of *K. pneumoniae* are presented in (Table 02 and Figure 05 to 09).

Table 02: Biochemical characters of *Klebsiella pneumoniae* isolates

Sr. No.	Biochemical test	Results
1	Catalase	Positive
2	Voges Proskauer	Positive
3	Oxidase	Negative
4	Methyl red	Negative
5	Indole	Negative

Figure 04: Active bubble formation showing positive catalase test

Figure 05: No color change showing negative oxidase test

Figure 06: No color change showing negative methyl red test

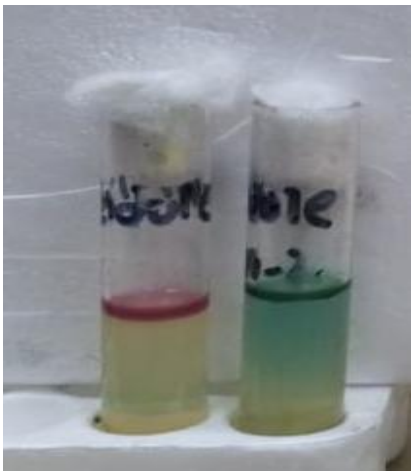

Figure 07: Representation of indole test for *K. pneumoniae*

Figure 08: Change in color showing positive Voges Proskauer test

Molecular confirmation of *K. pneumoniae* isolates

Total (n= 24) isolates which were identified on basis of morphology and biochemical characteristics were confirmed by PCR. The 657bp band for the KP16S gene was observed for all the *K. pneumoniae-positive* isolates as shown in (Figure 10)

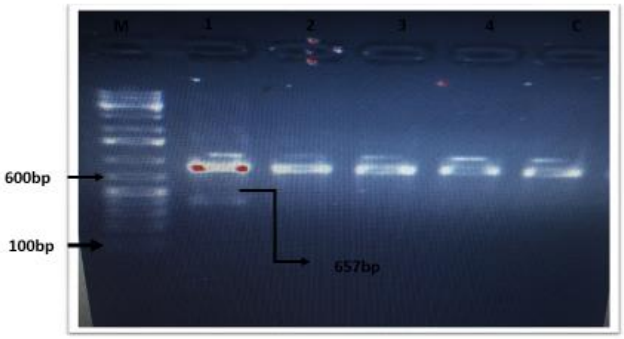

Figure 09: Gel showing 657bp band of KP_16S gene of *Klebsiella pneumonia*

Antibiotic susceptibility profiling

Antibiotic susceptibility testing of isolates was carried out on Muller Hinton agar using Kirby-Bauer disc diffusion assay. A total 24 isolates were exposed to antibiotics sensitivity testing. Zone of inhibition of the tested antibiotics were interpreted as said by Clinical Laboratory Standard Institute (CLSI 2020) guidelines. The isolates resistant to Ampicillin (AMP) were 14 (58.34%), Cefotaxime (CTX) 13 (54.17%), Ceftriaxone (CRO) 13 (54.17%), Ceftazidime (CAZ) 13 (54.17%), Amoxicillin/clavulanic acid (AMC) 11 (45.83%), Gentamicin (CN) 11 (45.83%), Amikacin (AK) 11 (45.83%), Tazobactam (TAZ) 11 (45.83%), Ciprofloxacin (CIP) 10 (41.67%), Meropenem (MEM) 7 (29.17%), Imipenem (IPM) 5 (20.83%). Overall highest susceptibility percentage was found for imipenem (16/24 66.67%) while highest resistance percentage was found for ampicillin (14/24 58.33%). Results for antibiotic susceptibility profiling of *K. pneumoniae* isolates have been presented in (Table 03 to 05) and (Figure 11 to 13).

Table 3: Antibiotic susceptibility pattern for *K. pneumoniae* isolates from beef

Antibiotic used	Resistance	Intermediate	Susceptible
Amoxicillin clavulanic acid (AMC)	11 (45.83%)	3 (12.50%)	10 (41.67%)
Gentamicin (CN)	11 (45.83%)	2 (8.33%)	11 (45.83%)
Ceftriaxone (CRO)	13 (54.17%)	0	11 (45.83%)
Ciprofloxacin (CIP)	10 (41.67%)	4 (16.67%)	10 (41.67%)
Ceftazidime (CAZ)	13 (54.17%)	3 (12.50%)	8 (33.33%)
Cefotaxime (CTX)	13 (54.17%)	3 (12.50%)	8 (33.33%)
Amikacin (AK)	11 (45.83%)	4 (16.67%)	9 (37.50%)
Imipenem (IPM)	5 (20.83%)	3 (12.50%)	16 (66.67%)
Meropenem (MEM)	7 (29.17%)	5 (20.83%)	12 (50%)
Tazobactam (TAZ)	11 (45.83%)	4 (16.67%)	9 (37.50%)
Ampicillin (AMP)	14 (58.34%)	1 (4.17%)	9 (37.50%)

Table 4: Antibiotic susceptibility pattern for *K. pneumoniae* isolates from fresh beef

Antibiotic used	Resistance	Intermediate	Susceptible
Amoxicillin clavulanic acid (AMC)	7 (46.67%)	1 (6.67%)	7 (46.67%)
Gentamicin (CN)	7 (46.67%)	1 (6.67%)	7 (46.67%)
Ceftriaxone (CRO)	8 (53.33%)	0	7 (46.67%)
Ciprofloxacin (CIP)	6 (40%)	3 (20%)	6 (40%)
Ceftazidime (CAZ)	8 (53.33%)	2 (13.33%)	5 (33.33%)
Cefotaxime (CTX)	8 (53.33%)	2 (13.33%)	5 (33.33%)
Amikacin (AK)	7 (46.67%)	3 (20%)	5 (33.33%)
Imipenem (IPM)	3 (20%)	2 (13.33%)	10 (66.67%)
Meropenem (MEM)	5 (33.33%)	2 (13.33%)	8 (53.33%)
Tazobactam (TAZ)	7 (46.67%)	2 (13.33%)	6 (40%)
Ampicillin (AMP)	9 (60%)	1 (6.67%)	5 (33.33%)

Table 5: Antibiotic susceptibility pattern for *K. pneumoniae* isolates from frozen beef

Antibiotic used	Resistance	Intermediate	Susceptible
Amoxicillin clavulanic acid (AMC)	4 (44.44%)	2 (22.22%)	3 (33.33%)
Gentamicin (CN)	4 (44.44%)	1 (11.11%)	4 (44.44%)
Ceftriaxone (CRO)	5 (55.55%)	0	4 (44.44%)
Ciprofloxacin (CIP)	4 (44.44%)	1 (11.11%)	4 (44.44%)
Ceftazidime (CAZ)	5 (55.55%)	1 (11.11%)	3 (33.33%)
Cefotaxime (CTX)	5 (55.55%)	1 (11.11%)	3 (33.33%)
Amikacin (AK)	4 (44.44%)	1 (11.11%)	4 (44.44%)
Imipenem (IPM)	2 (22.22%)	1 (11.11%)	6 (66.67%)
Meropenem (MEM)	2 (22.22%)	3 (33.33%)	4 (44.44%)
Tazobactam (TAZ)	4 (44.44%)	2 (22.22%)	3 (33.33%)
Ampicillin (AMP)	5 (55.55%)	0	4 (44.44%)

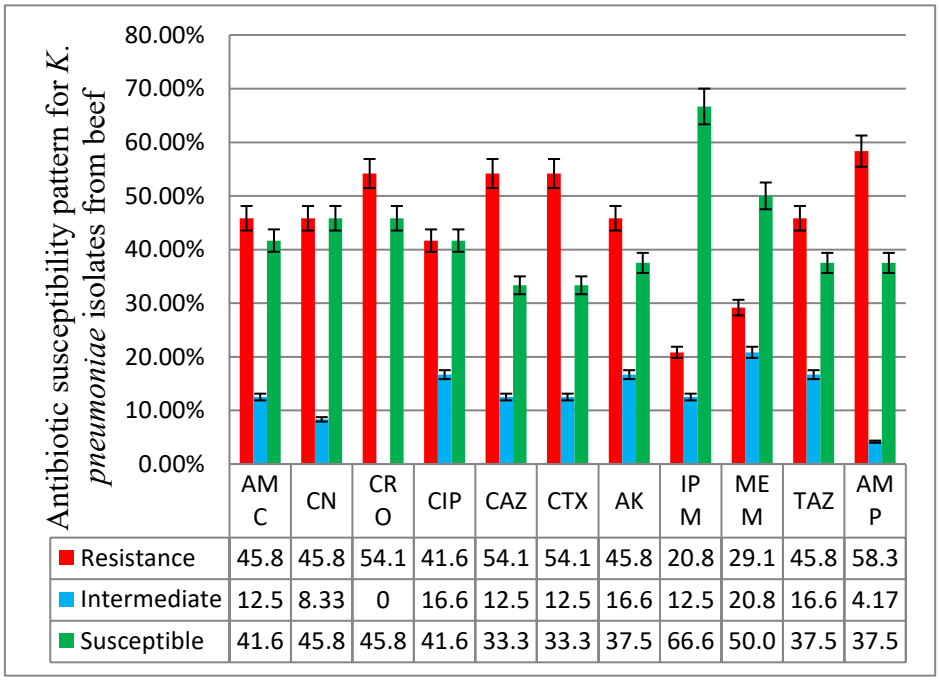

Figure 10: Graph presenting antibiotic susceptibility pattern for *K. pneumoniae* isolates from beef

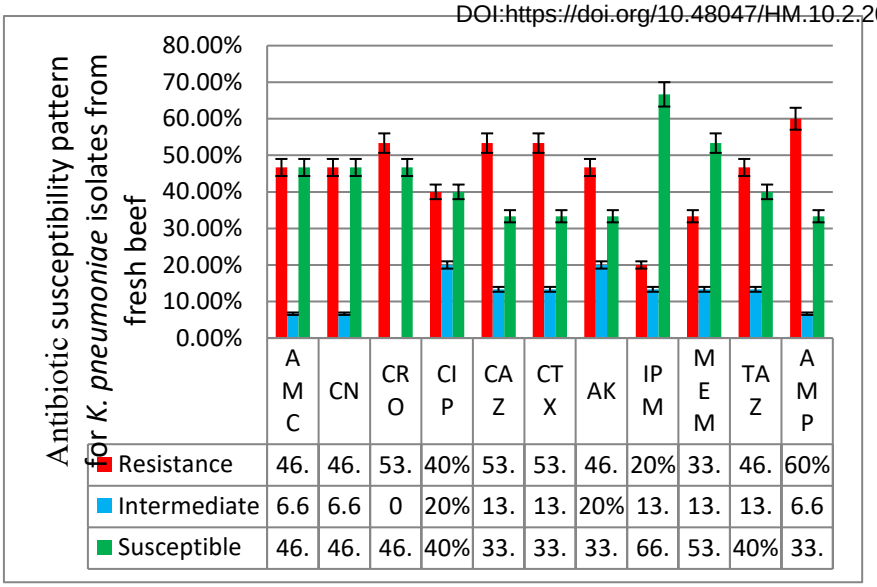

Figure 11: Graph presenting antibiotic susceptibility pattern for *K. pneumoniae* isolates from fresh beef

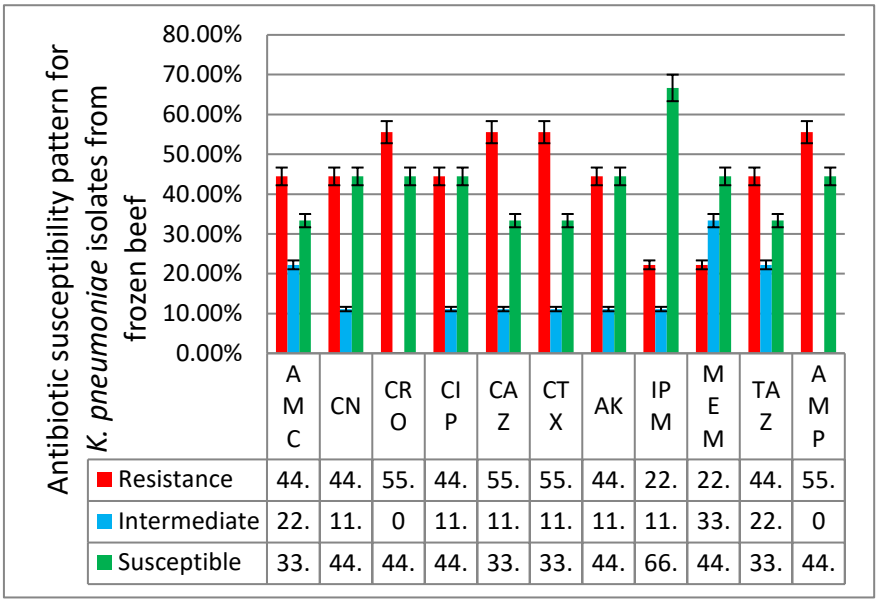

Figure 12: Graph presenting antibiotic susceptibility pattern for *K. pneumoniae* isolates from frozen beef

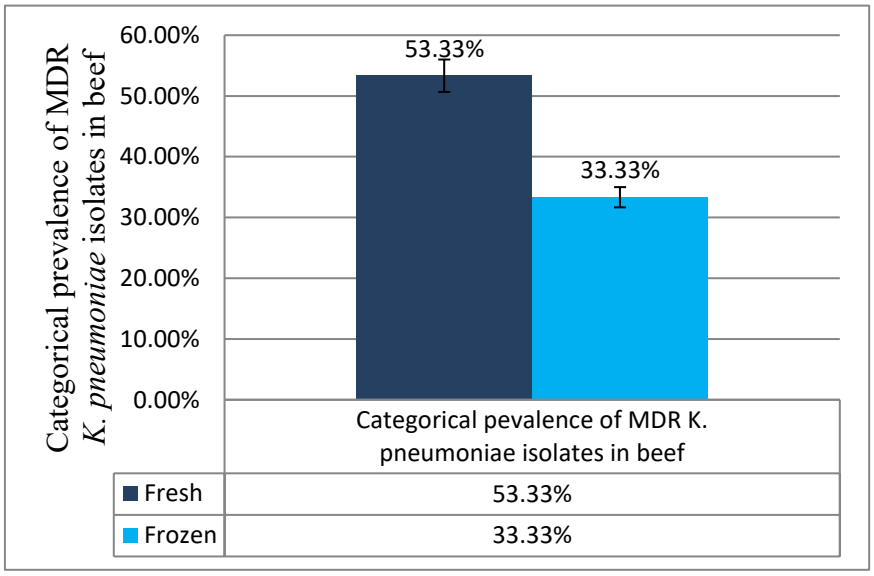

Figure 13: Graph presenting the categorical prevalence of MDR *K. pneumoniae* in beef

Molecular detection of ESBL genes

MDR isolates (n = 11) were processed further for the detection of ESBL genes. Highest prevalence was detected for CTX-M (36.36%) followed by OXA (27.27%) and TEM (18.18%) while none of the isolates was found positive for NDM. Multiple genes (n = 2) were detected in 1 isolate (9.09%) while 7 isolates (63.63%) harbored single gene and none of the genes was detected in 3 isolates (27.27%). Prevalence of ESBL genes in *K. pneumoniae* isolates has been presented in (Table 6 and Figure 15).

Table 6: Presence of ESBL genes in *K. pneumoniae* isolates

Gene name	No. of positive isolates	Percentage
bla_{CTX-M}	4	36.36%
Bla_{0XA}	3	27.27%
bla_{TEM}	2	18.18%
bla_{NDM}	0	0

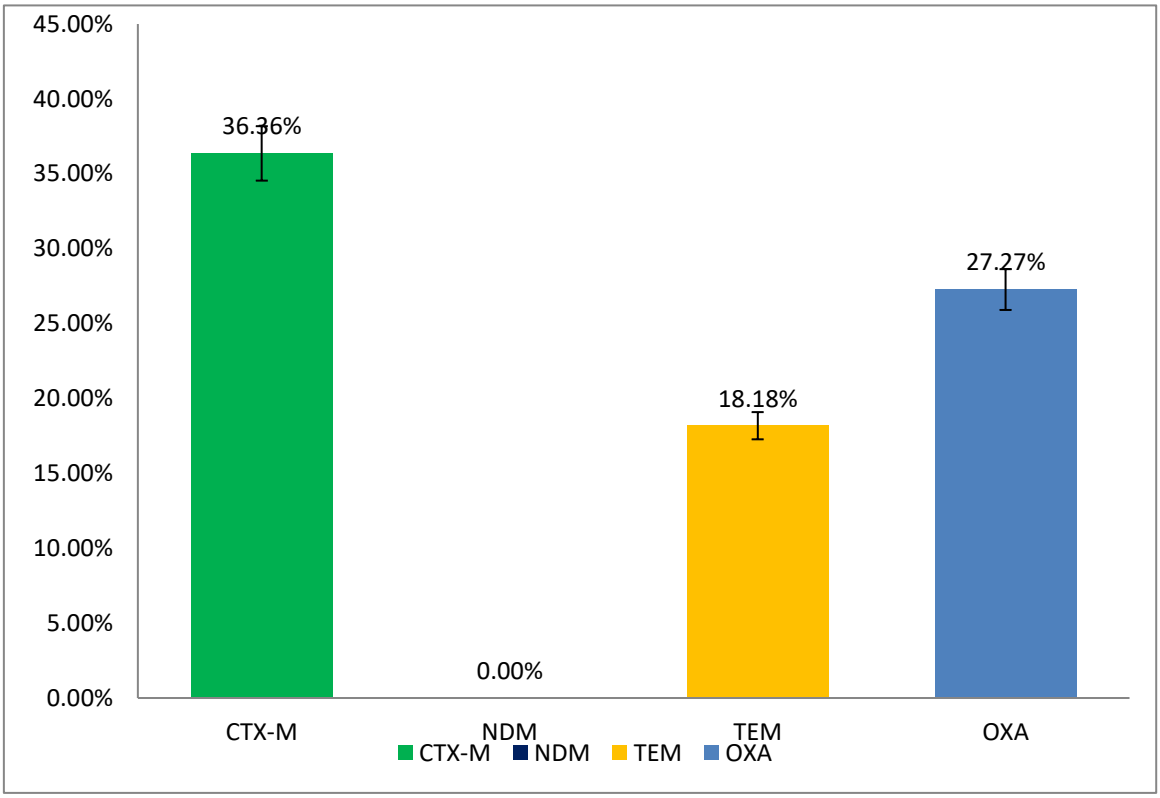

Figure 14: Presenting the prevalence of ESBL genes in *K. pneumoniae* isolates

Discussion

Klebsiella pneumoniae is a Gram-negative, lactose-fermenting bacillus with a conspicuous capsule that belongs to the *Enterobacteriaceae* family. *K. pneumoniae* is a common opportunistic pathogen found in the mouth, skin, and intestines, as well as in hospital settings and medical gadgets.

Opportunistic *K. pneumoniae* primarily affects people who have reduced immune systems or have been damaged by prior diseases(Dong et al., 2022). *K. pneumoniae* is not commonly considered as a typical food-borne pathogen and the majority of research on food-borne strains focuses on *Escherichia coli, Salmonella*, and *Shigella* instead. As a result, it is difficult to get information on the frequency with which *K. pneumoniae* strains are found in retail food as well as information about their pathogenicity, characteristics, and antibiotic resistance (Theocharidi et al., 2022). In this study, total of 100 beef samples (50 fresh and 50 frozen) were collected. Different techniques were used for the identification or confirmation of *K. pneumoniae.* After all the confirmatory tests 24 (24%) samples were confirmed positive for *K. pneumoniae*. This prevalence is less than the prevalence reported by (Rodrigues et al., 2023). They reported a 43.3% prevalence in chicken meat samples. The results of the present study were somehow consistent with the study conducted by(Junaid et al., 2022), who reported 22% occurrence of *K. pneumonia* from meat sample. Another study showed a 57% frequency of *K. pneumoniae* in beef samples described by (Qutub et al., 2022).In the present research occurrence of *K. pneumonia* was high in fresh beef samples (30%) and less in frozen beef samples (18%). Research conducted by (Guo & White, 2016) also reported a high prevalence of bacteria in fresh raw chicken 13.8%, and less in frozen samples 11.4%. Similarly, a Study conducted by (Gelbíčová et al., 2019) described that the prevalence of *K. pneumoniae* was higher in fresh meat than in frozen meat because mostly frozen meat was hygienically packed and in freezing conditions growth of bacteria retards. In this study, the Kirby-Bauer disc diffusion test was employed to evaluate the susceptibility pattern displayed by various drugs against *K. pneumoniae* isolates according to CLSI (2020) standards. The following drugs were used to test *K. pneumoniae's* sensitivity to antibiotics. Amoxicillin clavulanic acid (AMC), Gentamicin (CN), Ceftriaxone (CRO), Ciprofloxacin (CIP), Ceftazidime (CAZ), Cefotaxime (CTX), Amikacin (AK), Imipenem (IPM), Meropenem (MEM), Tazobactam (TAZ), and Ampicillin (AMP) were among the antibiotics used to treat pneumoniae isolates from beef. The least resistance was observed by Imipenem (20.83%), and Meropenem (29.17%), while the rest of the drugs showed high resistance. Montso et al., (2019) reported high resistance pattern of Cephalothin (100%), Ertapenem (66.7%), Ceftazidime (66.7%), Aztreonam (66.7%) Cephalothin (100%), Ertapenem (66.7%), Ceftazidime (66.7%), Aztreonam (66.7%) but Amoxicillin (66.7%) as well as Cefoxitin (33.3%) Cefotaxime (33%), Cefepime (33.3%) and Piperacillin (33.3%) showed least resistance against *K. pneumoniae.* Another study conducted by Promite et al., (2017) showed the resistance against various classes of antibiotics in the beef sample. They also reported the least resistance towards Carbapenem (20%). The findings of current study and their comparison with previously conducted studies showed that carbapenem class antibiotics showed the least resistance against *K. pneumoniae* so these are the best choices for the treatment of *K. pneumoniae* in beef. Antibiogram analysis for *K. pneumoniae* isolates from fresh explained the resistance pattern against antibacterial and they also showed least resistance against Imipenem (20%), Meropenem (33.33%). Study conducted by Najjuka et al., (2016) also showed least resistance towards Ceftriaxone (2.9%), Cefotaxime (2.2), Ceftazidime (2.5%), Ciprofloxacin (10.7%), Gentamicin (10.7%), as well as meropenem showed (100%) susceptibility pattern against *K. pneumoniae*. Antibiotic susceptibility test for *K. pneumoniae* isolates from frozen samples showed the resistance pattern against antibacterial drugs was Amoxicillin clavulanic acid (44.44%), Gentamicin (44.44%), Ceftriaxone (55.55%), Ciprofloxacin (44.44%), Ceftazidime (55.55%), Cefotaxime (55.55%), Amikacin (44.44%), Tazobactam (44.44%) and Ampicillin (55.55%) while least resistance was observed by Imipenem (22.22%), Meropenem (22.22%).Hayati et al., (2019)explained the susceptibility pattern in frozen meat as Ampicillin (100%),

amoxicillin (100%), oxytetracycline (90.9%), doxycycline (54.5%), ciprofloxacin (27.3%), enrofloxacin (18.2%), colistin (9.1%) and gentamicin (0%).

In current investigation we concluded that carbapenem class (meropenem, Imipenem) found to be least resistance against *K. pneumoniae*either isolated from fresh or frozen meat samples as compared to other antimicrobial drugs so the treatment of choice is carbapenem. In this study, total MDR isolates were recorded high (53.33%) in fresh beef samples while low in frozen samples (33.33%). Similarly, previously conducted study by Hu et al., (2021) showed high prevalence of MDR in fresh while low in frozen samples.

ESBL genes investigated in this study included CTX-M (36.36%), OXA (27.27%), TEM (18.18%), and none of the NDM was detected. Multiple genes (n = 2) were detected in 1 isolate (9.09%) while 7 isolates (63.63%) harbored a single gene and none of the genes was detected in 3 isolates (27.27%). A similar study was carried out to show the prevalence of TEM genes detected in 61 (57.55%) isolates. 49 (46.23%) isolates harbored CTX-M genes, and 25 (23.58%) carried genes of the SHV family by Abdallah et al., (2015), another study conducted by Bilal et al., (2021) explained 23 (18.4%) of the *K. pneumoniae* isolates carried the *bla*NDM-1 gene, making a total of 34 (27.2%) carbapenemase producers. Additionally, the genes for the additional carbapenemase, *bla*IMP-1 (7.2%), *bla*VIM-1 (3.2%), and *bla*OXA-48 (2.4%), were found.

In conclusion, it is established that fresh beef products have a higher frequency of *K. pneumoniae* than frozen beef. According to this data, *K. pneumoniae* is prevalent in humans and has a significant ratio in living animals. Antibiotic resistance is emerging constantly. Additionally, the majority of the identified strains included genes for virulence and carbapenemase resistance, which suggested that these infections posed a serious threat to human health and had a high potential for pathogens. The current understanding showed the possible risk of *K. pneumoniae* in retail food safety, food hygiene, and public health will be aided by the study's results. To further describe the prevalence and resistance profile of food-borne *K. pneumoniae* strains, more in-depth study is required.

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