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Antibiotic Resistance and Molecular Characterization of ESBL and MBL-Producing *Klebsiella pneumoniae* from Chickens

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ABSTRACT

Due to its increasing prevalence, antimicrobial resistance (AMR) is one of the biggest public health concerns. The production of extended-spectrum beta-lactamases (ESBLs) is a major cause of resistance in gram-negative bacteria like *Klebsiella pneumoniae*, making them resistant to penicillin's and cephalosporins. This work focused on analyzing the morphological and molecular features of Beta-Lactamase (ESBL) and Metallo-Beta-Lactamase (MBL) producing *K. pneumoniae* -isolates from poultry sources. The strains were obtained from different sources in three different cities of Pakistan. authors analyzed the antibiogram profiles of the samples and investigated their profiles of resistance genes. The results demonstrate that *K. pneumoniae* isolates were found in 49% (49/100) of the poultry samples. Specifically, 15 (75%) were collected from Chicken ceca, 10 (50%) from the heart, 10 (50%) from the liver, 9 (45%) from the lungs, and 5 (25%) from the trachea. Following incubation on MacConkey agar, colonies that were suspected to be *K. pneumoniae* were identified using phenotypic testing. The results showed that out of the 49 strains tested, 26 (53%) were found to be multidrug-resistant (MDR), 21 (43%) were positive for ESBL, and 12 (25%) were positive for MBL according to the double-disk synergy test (DDST). These strains exhibited resistance to augmentin (92%), ciprofloxacin (65%), ceftazidime (59%), piperacillin/tazobactam (59%), cefotaxime (57%), doxycycline (55%), ceftriaxone (53%), aztreonam (49%), meropenem (46%), and imipenem (46%). The genotypic prevalence of blaCTX-M-1 was 30%, blaIMP was 14%, and blaVIM was 8%. The occurrence of ESBL and MBL-producing bacteria in chicken samples is substantial, and there is a consistent rise in levels of antibiotic resistance. Hence, it is important to be careful when prescribing antibiotics and ensure they are used effectively."

A graphical Presentation of the Abstract is attached with a supplementary file

Keywords: Extended-spectrum beta-lactamase, Metallo beta-lactamase, Multidrug-resistant, antimicrobial, Double disc synergy test.

INTRODUCTION

Klebsiella species are frequently found in the intestines of poultry but might lead to contamination if the immune system of an infected bird is weakened (Younis *et al*., 2016). *K. pneumoniae* is a notable pathogen that is accountable for several healthcare-associated infections (Paczosa *et al*., 2016). Antibiotic resistance poses a substantial public health concern. Enterobacteriaceae that produce ESBLs are a significant worldwide concern to human health among multi-drug resistant infections (Leena *et al*., 2020). ESBLs are enzymes that are encoded by plasmids and provide resistance to third- and fourth-generation cephalosporins, as well as monobactams. ESBLs confer resistance to many beta-lactam antibiotics but remain susceptible to carbapenems and cephamycin. *K. pneumoniae* is the primary bacterium linked to these enzymes. β-lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam effectively counter ESBL activity, restoring antibiotic efficacy. (Paterson *et al*., 2005). The global emergence of -ESBL producing microbes is a significant and growing concern, particularly about hospital-acquired illnesses. These enzymes are particularly important in *K. pneumoniae* and *E. coli*, as these bacteria can produce a wide range of them. CTX-M genes are globally recognized as one of the most important types of ESBL, exhibiting a higher prevalence compared to other ESBLs (Hui *et al*., 2019). The ESBL encompasses the TEM and SHV β-lactamase families, but more recent descriptions have been made for other β-lactamases such as CTX-M, PER, and KPC (Leena *et al*., 2020). The global rise of MDR strains of *K. pneumoniae* poses a significant

public health concern for underdeveloped countries with limited resources. The isolates have multiple ways to avoid the effects of β-lactam medicines, including penicillin, cephalosporins, and carbapenems. These treatments are commonly used in both routine and critical care settings (Aminul *et al*., 2021). According to the China Antimicrobial Surveillance Network data shows *K. pneumoniae* resistance to carbapenems rose from 3.0% in 2005 to 23.8% in 2021, with MDR strains increasing from 0.3% to 3.5% (2008–2016). Resistance rates were higher in East China, but public health surveillance was limited during COVID-19. Understanding MDR mechanisms is crucial for better control (Yang *et al.,* 2023). ESBL-producing organisms can be detected by adding supplements to the media and then confirming their presence through phenotypic testing utilizing a combination of cefotaxime and ceftazidime with clavulanic acid, as well as ESBL E-strips. Diverse genetic processes have been linked to the transfer of genes that confer resistance to antimicrobial agents. *K. pneumoniae* has emerged as a significant public health threat due to the rise of multi-drug resistant (MDR) strains, particularly those associated with hospital outbreaks. Data from the European Antimicrobial Resistance Surveillance Network in 2019 revealed that over one-third (36.6%) of *K. pneumoniae* isolates were resistant to at least one major antimicrobial group, including fluoroquinolones, third-generation cephalosporins, aminoglycosides, and carbapenems. This alarming resistance pattern highlights the urgent need for effective strategies to combat these MDR strains and mitigate their impact on healthcare systems (Worku *et al*., 2024). Therefore, our study helps to enable targeted therapy to save lives and reduce the empirical use of antibiotics and ultimately the health and economic burdens of antibiotic resistance. Livestock significantly influences the widespread dispersion of antimicrobial-resistant and MBL-producing *K. pneumoniae* strains in food-producing animals. This poses a concerning threat to the emergence of infectious diseases in animals. Excessive utilization and inappropriate administration of antibiotics have significantly contributed to the emergence of antibiotic resistance. Excessive or improper use of antibiotics can lead to bacterial adaptation and the development of resistance to these drugs. This phenomenon renders infections more resistant to treatment, resulting in prolonged illnesses, increased hospitalizations, and an elevated likelihood of mortality (Ventola *et al.,* 2015). The significant occurrence of ESBL and MBL-producing bacteria detected in poultry samples is concerning, given the escalating trend of antibiotic resistance. This highlights the crucial importance of cautious antibiotic prescribing and the proper utilization of these drugs. Efficient management of antibiotics is crucial in order to regulate and diminish the transmission of microorganisms that have developed resistance. Therefore, our study helps to enable targeted therapy to save lives and reduce the empirical use of antibiotics and ultimately the health and economic burdens of antibiotic resistance.

MATERIALS AND METHODS

Sample Collection and Processing: All samples used in the study were collected from poultry farms, butcher shops, and local meat retailers in Islamabad, Rawalpindi, and Wah Cantt. A total of 100 poultry samples including (trachea, lung, liver, chicken ceca, heart). Samples were placed in sterile plastic bags and transported to the National Veterinary Laboratory Islamabad for bacteriological study. The poultry samples were collected at the poultry farms, butchers and local meat retailers for the isolation of *K. pneumoniae*. All samples were collected from Islamabad, Rawalpindi and Wah cantt were used in the study. The study was conducted between October 2022 and May 2023.

Identification of *K. pneumoniae* isolates: For the isolation of *Klebsiella pneumonia,* chicken samples (25 g) were placed in pre-enriched 10ml buffered brain heart infusion (BHI) broth for 18-24 h at 37°C. To assay for bacterial growth, the poultry samples were streaked onto MacConkey agar plates and incubated at 37°C for 18-24 h. All isolates were confirmed to be K. pneumoniae based on morphological characterization, Gram staining pattern (ASM 2005; Rawy et al., 2020), and biochemical tests (catalase, indole test, methyl red, vogues Proskauer and citrate utilization test) and further confirmation of K. pneumoniae strains at the species level was accomplished by the analytical profile index API 20E kit (Atlas et al., 1995).

Antimicrobial Susceptibility Testing: The antimicrobial susceptibility profiling of the K. pneumoniae strains contrary to antibiotics was checked through the technique of the Kirby and Bauer disc diffusion method according to the references of the Clinical Laboratory Standard Institute guidelines 2018 (Table 1) (Ejikeugwu et al., 2016). A sterile cotton swab was used to cultivate the strains on Mueller-Hinton agar (MHA) plates. The swabbed Mueller Hinton agar plates were cultured with ten different antibiotics: Amoxicillin+clavulanic acid (10µg/20µg), piperacillin/tazobactam (100µg/10µg), ceftazidime (30µg), ceftriaxone (30µg), aztreonam (30µg), doxycycline (30µg), cefotaxime (30µg), ciprofloxacin (5µg), imipenem (10µg), and meropenem (10µg). After a full day plates were checked out for the zone of inhibition and the diameter of zones in mm which was read as sensitive, intermediate, and resistant a ruler (Iroha et al., 2017). Plates were examined for the zone of inhibition and zone diameter in millimetres after a full day. The zones were interpreted as sensitive, intermediate, and resistant using a ruler (Iroha et al., 2017). According to Cordova et al. (2023), microorganisms that are resistant to several treatment categories are referred to as multidrug-resistant (MDR). The 2018 CLSI guidelines were used to interpret all antibiotic results.

Table 1: CLSI 2018 guidelines for Zone of Inhibition of antimicrobial agents used for *K. pneumoniae*

S. No	*Antimicrobial Agents*	*Resistance*	*Intermediate*	*Sensitive*
1	*CIPROFLOXACIN/ CIP*	≤15 mm	16-20 mm	≥21 mm
2	*CEFOTAXIME/CTX*	≤22 mm	23-25 mm	≥26 mm
3	*CEFTAZIDIME/CAZ*	≤17 mm	18-20 mm	≥21 mm
4	*DOXYCYCLINE/DXT*	≤10 mm	11-13 mm	≥14 mm
5	*CEFTRIAXONE/CRO*	≤19 mm	22-20 mm	≥23 mm
6	*AMOXICILLIN+CLAVULANIC ACID/AUG*	≤13 mm	14-17 mm	≥18 mm
7	*AZTREONAM/ATM*	≤17 mm	18-20 mm	≥21 mm
8	*MEROPENEM/MEM*	≤19 mm	22-20 mm	≥23 mm
9	*IMIPENEM/IMP*	≤19 mm	22-20 mm	≥23 mm
10	*PIPERACILLIN/TAZOBACTAM/TZP*	≤17 mm	18-20 mm	≥21 mm

Phenotypic detection of ESBL: The confirmation of ESBL was done by phenotypic confirmation methods Double disc synergy test (DDST). MHA plates were inoculated with 0.5 McFarland-matched test inoculums. On the inoculated plate augmentin disc was placed in the middle of the MHA plate. Aztreonam disc along with three discs of third-generation cephalosporins (Ceftriaxone, Cefotaxime, Ceftazidime) was placed at a space of 20 mm (center to center) from AUG disc and placed the plate in the incubator. For those inoculums that exhibited an enhanced zone of inhibition, the synergism between Amoxicillin/clavulanic acid and cefotaxime or ceftazidime was identified as confirmed ESBL producers. The synergy was an indication of the ESBL positive (Marjani et al., 2013).

Phenotypic detection of MBL: The confirmation of MBL production was done by phenotypic confirmation methods Double disc synergy test. In imipenem EDTA-DDST isolated strain were inoculated in test tube having 5 milliliter of sterile peptone water and place the tubes in incubator at 37ºC for 30 minutes to obtain a turbidity of approximately 0.5 McFarland's standard which was measured with a turbidity meter. Lawn culture was done on the surface of an MHA plates. An imipenem/meropenem (10µg) disc was positioned on the surface of MHA plate center to center having a distance of 20 mm from a blank disc of filter paper containing 0.5 M EDTA. The plate was placed in the incubator for 16 to 18 hours. The occurrence of a synergistic inhibitory zone between imipenem/meropenem and EDTA disc was regarded as MBL positive (Khosravi *et al.,* 2013).

Molecular characterization of *K. pneumoniae* isolates:

DNA extraction: The microbial genomic DNA was extracted by the boiling method. A fresh bacterial colony (6 to 8) was suspended in 400 microliter of double distilled water heat for 10 min at 95°C a hot plate and cooled down the tubes. After 3000 rpm centrifugation for 5 minutes, DNA containing supernatant was transferred to a sterile Eppendorf tube (Ahmed *et al*., 2017). DNA quantitative was done by using spectrophotometer, the Nanodrop system 2000, spectrophotometer (Thermo Scientific) by assessing the ODs (optical density) of the extracted DNA samples which were taken at 260/280 nm.

The genotypic identification (PCR): Revealing of ESBL (blaCTX-M-1) and MBL (blaVIM and blaIMP) Polymerase chain reaction was conducted for the isolates. To determine the blaCTX-M-1, blaIMP and blaVIM genes, PCR was conducted by using the following primers (Table 2). Polymerase chain reaction amplification was done with a 25 µL total reaction volume. Conditions which was followed for the amplification of blaCTX-M-1, blaIMP, and blaVIM for thermal cycling.

Assessment of Amplified Gene by Using Agarose Gel Electrophoresis: Analysis of Amplicons was done on agarose gel (1.5%) with appropriate 100bp DNA ladder Thermo Scientific) to detect amplified products.

Gel Electrophoresis and Gel Documentation: The agarose gel (0.5 grams) was combined with 35 milliliters of 1X TBE in a graduated flask to prepare a 1.5% gel. The solution was heated in a microwave for one minute to fully dissolve the agarose. It was then allowed to cool before adding 3 microliters of ethidium bromide, which was thoroughly mixed into the solution. Subsequently, the solution was allowed to cool to ambient temperature for five minutes. The solution was carefully poured into the gel caster, and the combs were thereafter inserted. The solution was left to harden at room temperature for 15-20 minutes. Once the gel had solidified, the combs were delicately removed without causing any damage to the wells. The gel caster was placed into the gel tank, and a solution of 1X TBE (running buffer) was added. Before inserting the samples into wells, the samples were subjected to centrifugation for 30 seconds to separate the components. 6µl of DNA (sample) and 2µl of 6X loading dye (Thermo Scientific) were mixed and loaded into the wells using sterile micropipettes. In the initial well, a DNA ladder of 100 base pairs was introduced to restrict the size of the PCR product that was amplified. The gel was electrophoresed for 55 minutes at a constant voltage of 120 volts using APELEX PS 3002. The bands were transferred from the negative electrode to the positive electrode. Following gel electrophoresis, the gel band was examined using a Gel Documentation System illuminated by UV light.

Table 2. Primer sequence used for the genotyping of bla CTX-M 1, bla VIM and bla IMP genes

Gene	*Primers*	*Sequence*	*Product size (bp)*	*Reference*
bla CTX-M-1	Forward primer	AAAAATCACTGCGCCAGTTC	**415**	Khan et al., 2010
	Reverse primer	AGCTTATTCATCGCCACGTT		
bla IMP	Forward primer	GGAATAGAGTGGCTTAATTCTC		
	Reverse primer	CCAAACCACTACGTTATCT	188	Farhan et al., 2019
bla VIM	Forward primer	GATGGTGTTTGGTCGCATA		
	Reverse primer	CGAATGCGCAGCACCAG	390	Farhan et al., 2019

RESULTS

Isolation of *K. pneumoniae* from Poultry Samples: Out of the 100 samples that were obtained, 49 of them tested positive for isolates of *K. pneumoniae*. This bacterial species was identified by observing its colony morphology (pink colored colony with a mucoid appearance on MacConkey agar), Gram's reaction (Gram negative), motility test (non-motile), and various biochemical tests (including citrate test, indole test, catalase test, MR test, VP test) as well as the API 20E kit method.

Prevalence of *K. pneumoniae* in Various Organs of Chicken: A total of 100 chicken samples were collected from three cities of Pakistan (45 samples from Islamabad, 30 samples from Rawalpindi, and 25 samples from Wah Cantt) (Graph 1). The distribution of K. pneumoniae in various organs was as follows: 75% in chicken ceca, 50% in the heart and liver, 45% in the lungs, and 25% in the trachea. The chicken ceca had the highest occurrence of K. pneumoniae isolates. (Table 3).

Graph 1. *K. pneumoniae* isolates from Islamabad, Rawalpindi & Wah Cant

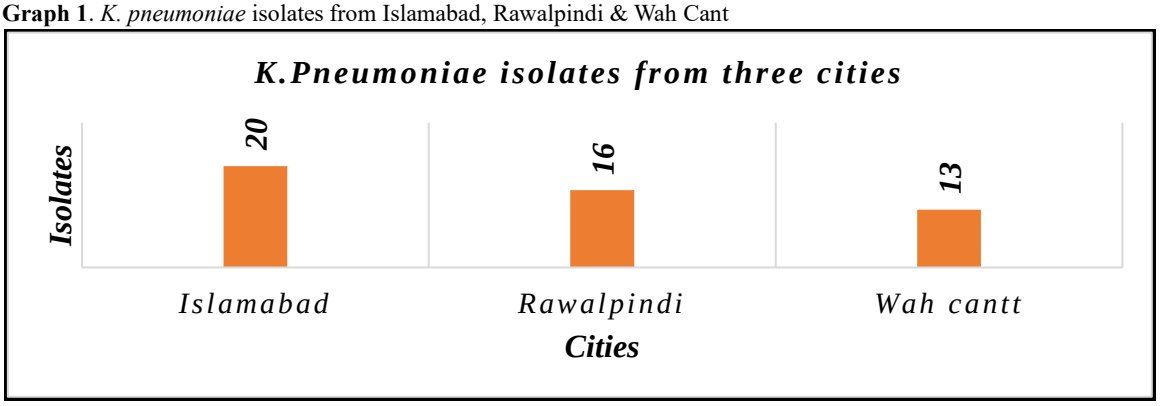


Table 3: Prevalence of *Klebsiella pneumoniae* Isolates in Different Chicken Organs.

S.No	*Organs*	*Total samples*	*K. pneumoniae isolates*	*Prevalence %age*
1	Trachea	*20*	*5*	*25*
2	Liver	*20*	*10*	*50*
3	Lungs	*20*	*9*	*45*
4	Chicken ceca	*20*	*15*	*75*
5	Heart	*20*	*10*	*50*
Total		*100*	*49*	*49*

Antimicrobial susceptibility testing. The susceptibility and resistance of all 49 verified K. pneumoniae isolates were assessed by testing them against different antibiotic. The zone of inhibition measurements for these antimicrobial agents were validated according to the guidelines set by the CLSI. The results showed a clear resistance to augmentin (92%), ciprofloxacin (65%), ceftazidime (59%), piperacillin/tazobactam (59%), cefotaxime (57%), doxycycline (55%), ceftriaxone (53%), aztreonam (49%), meropenem, and imipenem (46%). Conversely, aztreonam exhibited the highest sensitivity at a rate of 41% (Table 4).

Table 4. Zone of Inhibition for Resistance, Intermediate & Sensitive Values of Antimicrobial Agents According to CLSI guideline

S.No	*Antimicrobial Agents*	*Conc.*	*Resistance*		*Intermediate*		*Sensitive*	
			No	*%*	*No*	*%*	*No*	*%*
1	*CIPROFLOXACIN/ CIP*	5µg	*32*	*65*	*6*	*12*	*11*	*23*
2	*CEFOTAXIME/CTX*	30µg	*28*	*57*	*7*	*14*	*14*	*29*
3	*CEFTAZIDIME/CAZ*	30µg	*29*	*59*	*9*	*18*	*11*	*23*
4	*DOXYCYCLINE/DXT*	30µg	*27*	*55*	*8*	*16*	*14*	*29*
5	*CEFTRIAXONE/CRO*	30µg	*26*	*53*	*7*	*14*	*16*	*33*

6	*AMOXICILLIN+ CLAVULANIC ACID/AUG*	10µg/ 20µg	*45*	*92*	*2*	*4*	*2*	*4*
7	*AZTERONAM/ATM*	30µg	*24*	*49*	*5*	*10*	*20*	*41*
8	*MEROPANAM/MEM*	10µg	*23*	*46*	*7*	*14*	*19*	*36*
9	*IMIPENAM/IMP*	10µg	*23*	*46*	*7*	*14*	*19*	*36*
10	*PIPERACILLIN /TAZOBACTAM/TZP*	100µg /10µg	*29*	*59*	*8*	*16*	*12*	*25*

Multi-Drug Resistance (MDR): The susceptibility of 49 K. pneumoniae isolates to different antibiotics was investigated in this study. Of these, 26 isolates were found to have a multi-drug-resistant phenotype, meaning they were resistant to multiple drugs. Most of the multi-drug resistance strains were isolated from the chicken ceca 67%. In Table 5, the heart and trachea contributed 40% and 60%, respectively, whereas the liver and lung provided 56% and 40%. The 26 MDR isolates showed evidence of drug resistance. According to Table 5 and Graph 2, the prevalence of K. pneumoniae that was resistant to several drugs was 53%..

Detection of ESBL and MBL strains: ESBL (Chromogenic) media is a selective media for the growth of ESBL-producing microorganisms. The presence of greenish color colonies on chromogenic media was the phenotypic confirmation of ESBL-producing *K. pneumoniae* isolates. Out of 49 isolates, only 21 isolates were found ESBL-positive. ESBL production in *K. pneumoniae* isolates (n=49) was confirmed by the double disc synergy method as recommended by CLSI guideline, 2018. A total of 21 isolates were found ESBL positive out of 49 that showed synergy to cefotaxime, ceftazidime, or ceftriaxone and cefotaxime+ clavulanic acid, ceftazidime+ clavulanic acid. The prevalence of ESBL-producing *K. pneumoniae* was 43% (Table 5, Graph 2). MBL production in *K. pneumoniae* isolates (n=49) was confirmed by the double disc synergy method as recommended by CLSI guideline, 2018. A total of 12 isolates were found MBL positive out of 49 that showed synergy b/w imipenem and EDTA disk. The prevalence of MBL-producing *K. pneumoniae* was 25% (Table 5, Graph 2)

Table 5. Frequency and Percentage of ESBL, MBL and MDR-Producing *Klebsiella pneumoniae* Strain.

Sample Source	***ESBLs, MBL and MDR producing K. pneumoniae strains***						
	Total isolate	**Number of strains ESBLs**	**% of strains ESBLs**	**Number of strains MBLs**	**% of strains MBLs**	**Number of strains MDR**	**% of strains MDR**
Trachea	5	1	20	1	20	3	60
Heart	10	4	40	3	30	4	40
Liver	9	5	56	2	22	5	56
Chicken ceca	15	8	53	4	27	10	67
Lungs	10	3	30	2	20	4	40
Total	49	21	43	12	25	26	53

Polymerase chain reaction (PCR): *K. pneumoniae* isolates (ESBL=21 & MBL=12) were identified by PCR. Bacterial DNA was extracted by the boiling method and check DNA quality through nanodrop. The CTXM-1 gene was use to assign the 21 (15 ESBLs-positive and 6 ESBLs-negative) isolates to *K. pneumoinae* (Table 6, graph 3 and Figure 1). IMP and VIM gene was used to assign the 12 (7 MBLs-positive and 5 negative for bla_{IMP} and 4 MBLs-positive and 8 negative for bla_{VIM}) isolates to *K. pneumoinae* (Table 6, graph 3 and Figure 2,3)and Genotypic prevalence of $bla_{CTX\text{-}M\text{-}1}$ (30%) bla_{VIM} (8%) and bla_{IMP}(14%) genes among 49 strains of *K. pneumoniae* (Table 7).

Graph 2: Prevalence of MDR & Non-MDR, ESBL & Non-ESBL, MBL & Non-MBL Producing *K. pneumoniae* Isolates

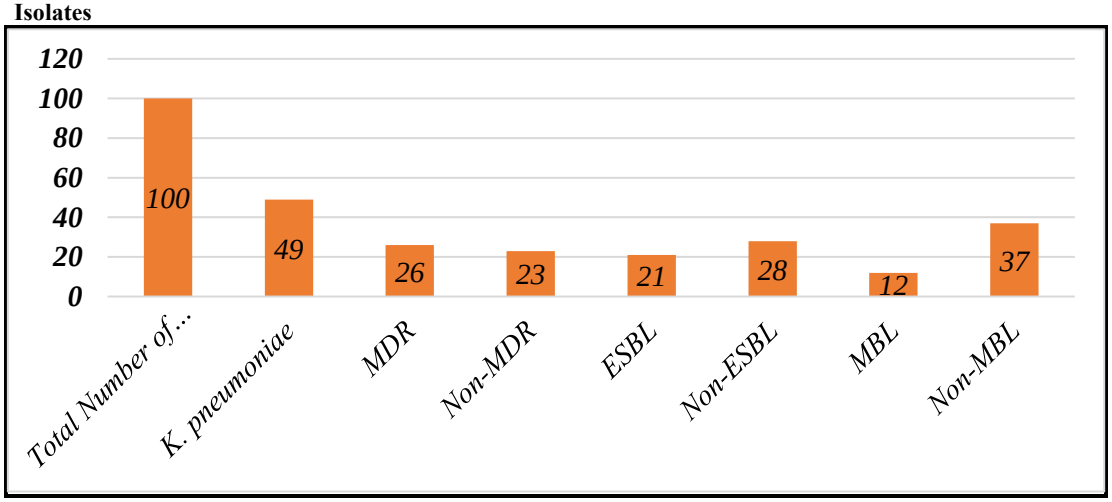


Table 6. Genotypic prevalence of bla_{VIM}, bla_{IMP} and $bla_{CTX\text{-}M\text{-}1}$ gene of *K. pneumoniae*

Sample source	Total Isolate	Phenotypic MBL Producing Strains	Genotypic MBLs producing strains		Phenotypic ESBsL Producing Strains	Genotypic ESBLs ($bla_{CTX\text{-}M\text{-}1}$ gene) producing strains
			bla_{VIM} gene	bla_{IMP} gene		
Trachea	5	0	0	0	1	1
Heart	10	3	1	2	4	3
Liver	9	2	1	1	5	3
Chicken ceca	15	5	2	3	8	6
Lungs	10	2	0	1	3	2
Total	49	12	4	7	21	15

Graph 3: Graph representation of genotypic prevalence of bla_{VIM}, bla_{IMP} and $bla_{CTX\text{-}M\text{-}1}$ gene of *K. pneumoniae*

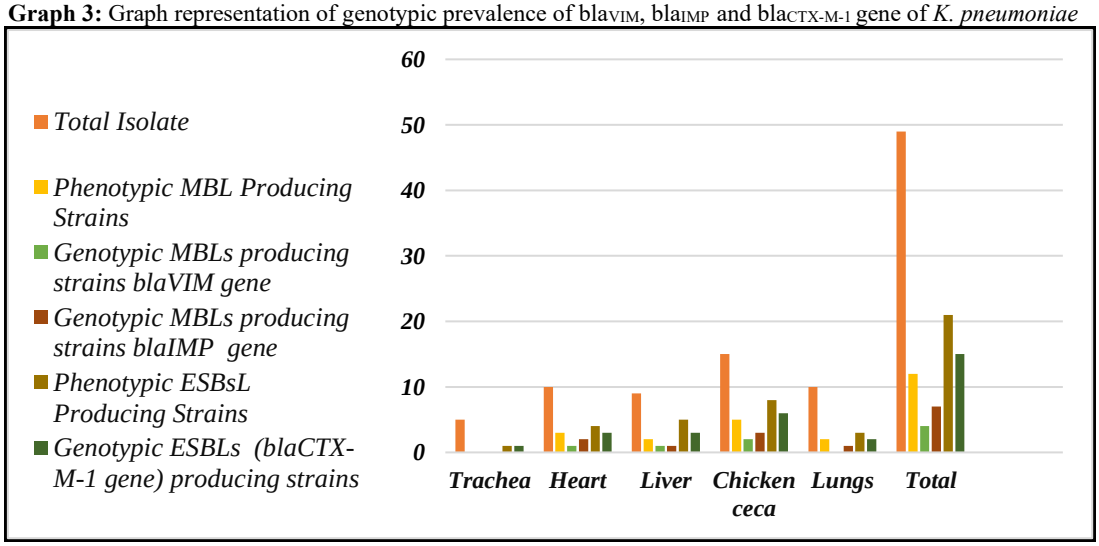
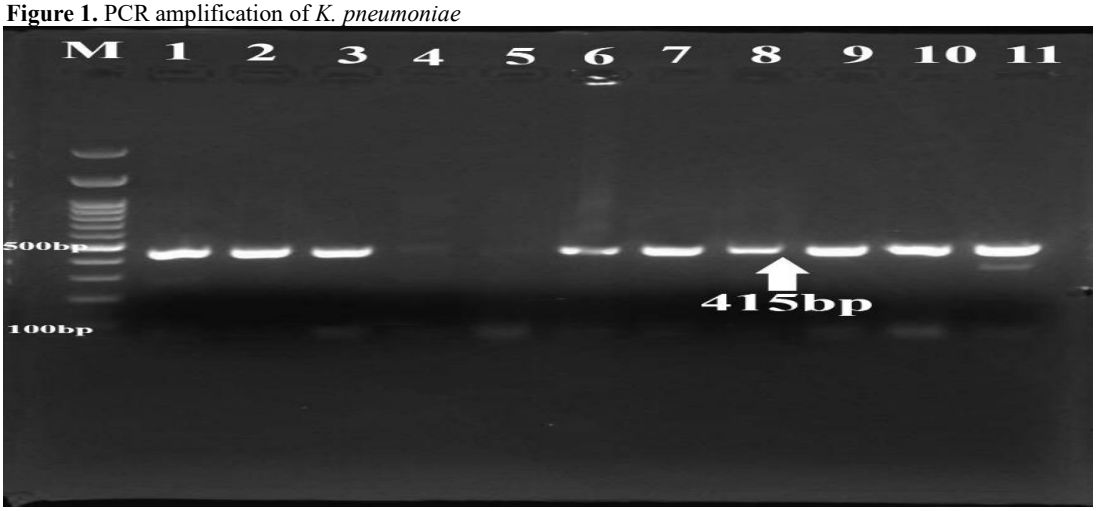
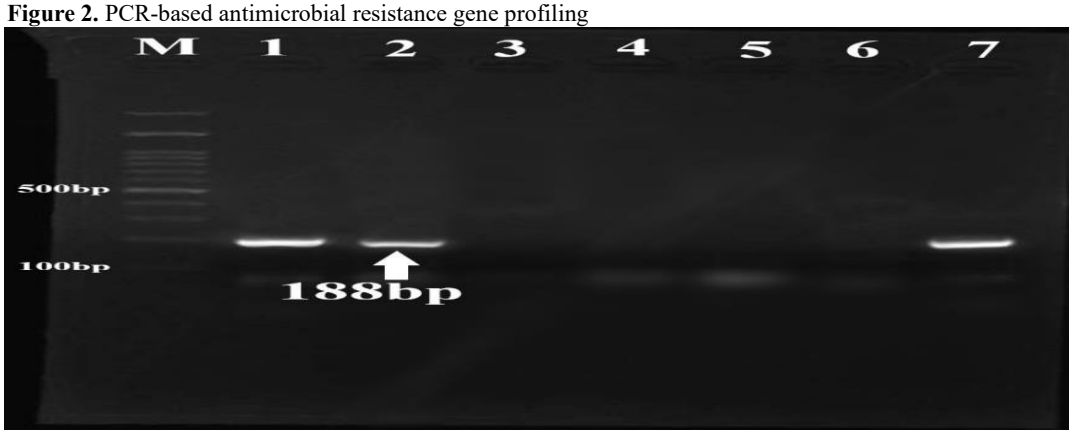


Figure 1. PCR amplification of *K. pneumoniae*



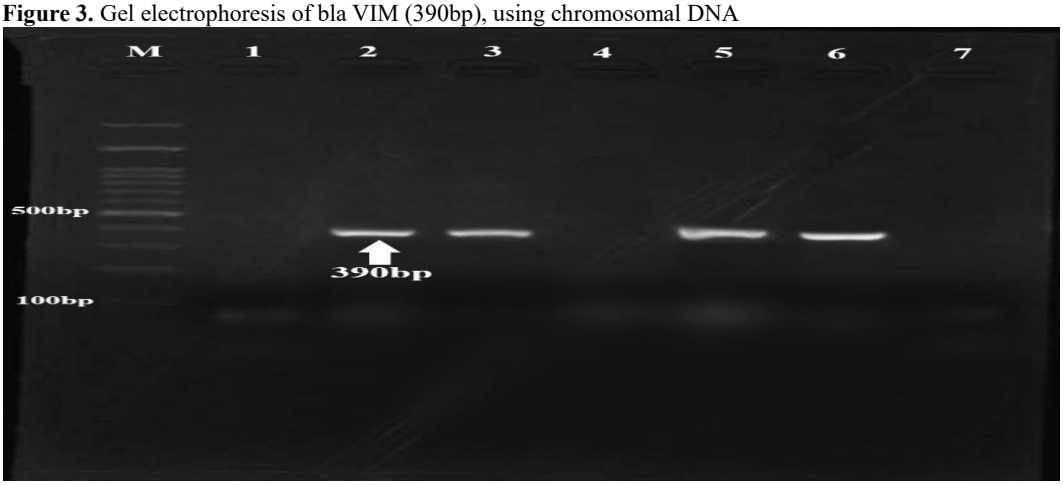
*M: 100 bp ladder
*Lane 1-3, 6-10 and 11 CTXM-1 415bp (ESBL) were representing samples positive for *K. pneumoniae*.
*Lane 4 and 5 were representing samples negative for *K. pneumoniae*

Figure 2. PCR-based antimicrobial resistance gene profiling



*M: 100 bp ladder
*Lane 1, 2, and 7 IMP 188bp (MBL) were representing samples positive for *K. pneumoniae*
* Lanes 3, 4 and 5, 6 represented samples negative for *K. pneumoniae*.

Figure 3. Gel electrophoresis of bla VIM (390bp), using chromosomal DNA



*Line M: 100 bp DNA ladder.
*Lane 2, 3, 5 and 6 positives for *K. pneumoniae*
*Lane 1, 4 and 7 negatives.

Table 7. Genotypic prevalence of $bla_{CTX\text{-}M\text{-}1}$, bla_{VIM} and bla_{IMP} genes among 49 strains of *K. pneumonia*

Name of gene	***Genotypic prevalence***
$bla_{CTX\text{-}M\text{-}1}$	30%

bla$_{VIM}$	8%
bla$_{IMP}$	14%

DISCUSSION

The global crisis of antibiotic resistance demands urgent attention, particularly in developing rapid, accurate, and reliable diagnostic methods. These tools are essential for the timely detection of antibiotic resistance, enabling healthcare providers to select appropriate treatments and curbing the spread of resistant bacteria. This study aims to emphasize the existing health issue associated with the consumption of high doses of antibiotics (Phillips *et al*., 2004). These antibiotics can cause significant harm to the stomach and contribute to the development of AMR over time, in addition to other potential side effects (Levy *et al*., 2013). This resistance is a significant global health threat, and one of its primary causes is the inappropriate use of antimicrobials in cattle. The study by Dandachi *et al*., (2018) suggests that chicken flesh may serve as a reservoir for Enterobacteriaceae containing ESBL, which can colonize and infect humans. Currently, AMR stands out as a prominent public health concern. Gram-negative bacteria mostly develop resistance through the development of ESBL (Mughini *et al*., 2020). Among others, *K. pneumoniae* is responsible for a diverse array of illnesses and is recognized as a primary cause of hospital-acquired infections in humans, such as urinary tract infections, pneumonia, septicemia and rhinoscleroma (Cunha *et al*., 2016). In this study, this bacteria was selected to test its resistance to ten different antimicrobial drugs, and the determination of the presence of the Extended Beta Lactamases (blaCTX-M-1) and Metallo Beta Lactamases (blaVIM and blaIMP) genes. *K. pneumoniae* has been isolated and identified from poultry birds, cattle, urine, and hospital (Abadullah *et al.,* 2016; Khan *et al*., 2016; Ajayi *et al.,* 011). The current investigation demonstrated that a substantial quantity of *K. pneumoniae* isolates with antibiotic resistance was found in various chicken samples, with the susceptibility of 49 isolates assessed against ten antimicrobial agents. The highest resistance was observed against augmentin (92%), followed by ciprofloxacin (65%), ceftazidime and piperacillin (59%), cefotaxime (57%), doxycycline (55%), and ceftriaxone (53%). Aztreonam showed a resistance rate of 49%, while meropenem and imipenem had a resistance rate of 46%. Conversely, aztreonam exhibited the highest sensitivity rate of 41%. This conclusion contradicts the findings of Shahcheragi, who found that *K. pneumoniae* showed resistance to piperacillin in 55% of cases, cefotaxime in 32% of cases, and ceftazidime in 31% of cases (Shahcheragi *et al*., 2007). In another study *K. pneumoniae* exhibited a 50% resistance to cefotaxime, 37% to ciprofloxacin, and 51% to ceftazidime. The bacteria also exposed decreased sensitivity to aztreonam and ampicillin (Feizabadi *et al*., 2006). The current investigation suggested that 49% of *K. pneumoniae* isolates were susceptible to aztreonam while Gharrah *et al*. (2017) specified resistance rates for *K. pneumoniae* as 49% to ceftriaxone, 40% to ceftazidime, 38% to aztreonam, and 49% to cefotaxime, indicating the percentage of isolates that were resistant, not susceptible. The ongoing analysis revealed a prevalence of 26 MDR *K. pneumoniae* strains, aligning with the 21 MDR *K. pneumoniae* infections described by Feizabadi. A recent study using the double disk synergy test (DDST) revealed that 42.8% of *K. pneumoniae* isolates tested positive for ESBL (Feizabadi *et al*., 2006), and the present findings are consistent with this data, showing that 44.5% of *K. pneumoniae* strains exhibited ESBL production. In a prior study conducted by Parveen *et al.* in 2011, it was reported that 97.2% of *K. pneumoniae* bacteria produced ESBL, which is higher than the percentage of ESBL production seen in the current study. In a study conducted by Charrakh *et al*. in 2011, it was stated that the production of ESBL in *K. pneumoniae* was found to be 21%, which is lower than the ESBL production rate seen in the current study. There have been reports of *K. pneumoniae*, which is produced by MBL, in poultry. The current study found that the prevalence rate of MBL in *K. pneumoniae* was 24.4%, which is similar to the findings of Borah *et al*. (2016) who reported MBL production in *K. pneumoniae* at a rate of 21.8% using the modified Hodge test and 24.52% using the Combine disk test. A study conducted in Pakistan by Humayun stated that 7.7% of *K. pneumoniae* isolates from PIMS hospitals were found to produce MBL. Another study by Kazemian *et al*. in 2019 reported a higher incidence of 43.3% of MBL-producing *K. pneumoniae*. The *K. pneumoniae* isolates that were shown to be phenotypically expressing ESBL and MBL were further evaluated to determine their genotypical resistance. The current investigation observed clear phenotypic resistance to augmentin (92%) and cefotaxime (57%). However, the prevalence of blaCTXM-1 was found to be 30%, which is consistent with the findings of Chaudhary *et al*., 2013 belong to India. who showed a prevalence of 37.33% for CTX-M in *K. pneumoniae*. The earlier study conducted by Zeynudin showed a prevalence rate of blaCTX-M in *K. pneumoniae* of 97.1%, which is relatively lower compared to the findings of the current study (Zeynudin *et al*., 2018). In Pakistan found that the percentage of blaCTX−M−1 was 65% (Abrar *et al*., 2019). *K. pneumoniae* strains that produce MBL were validated phenotypically using PCR. The 12 phenotypically MBL-generating *K. pneumoniae* isolates were subjected to PCR analysis to detect the presence of blaIMP and blaVIM genes. The current investigation found that the presence of blaIMP was detected in 7 of *K. pneumoniae* strains, whereas the presence of blaVIM was detected in 4 of the strains. A study conducted by Ansari revealed a prevalence of

blaVIM 4 and blaIMP 5, which was higher and equal than the prevalence reported in the current study (Ansari *et al*., 2021). A study conducted by researchers in Khartoum found that the prevalence of Verona integron Metallo beta-lactamase (VIM) was 38.9% and imipenem's (IMP) was 26.4% (Adam *et al*., 2018). In Uganda, Okoche reported the rates of VIM and IMP as 21 (10.7%) and 12 (6.1%) respectively (Okoche *et al*., 2015). In Tanzania, Mushi reported the frequencies of VIM and IMP as 34 (15%) and 28 (12%) respectively (Mushi *et al*., 2013). Based on current research, it appears that resistance to imipenem or meropenem is not the primary reason for the presence of MBL-producing isolates of *K. pneumoniae*. Other mechanisms of resistance may also be involved in the resistance to this antibiotic, as the majority of imipenem isolates that were not susceptible (46%) did not have the genes associated with resistance.

CONCLUSION

The study highlights rising antimicrobial resistance in poultry, particularly *Klebsiella pneumoniae*, with high levels of ESBL and MBL-producing strains and resistance genes (*blaCTX−M−1*, *blaIMP*, *blaVIM*). This threatens effective antibiotic treatment and increases the risk of resistant bacteria spreading to humans. Urgent action is needed through stringent antibiotic stewardship, regular resistance monitoring, and preventive measures. Further studies are essential to validate rapid resistance detection assays and combat this growing public health threat.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTION

SA, QF. Study conception and design, data acquisition and analysis, interpretation, drafting, critical revision, final approval. AN: Data analysis, data acquisition, drafting, critical revision, interpretation, and final approval. SM. Data analysis, data acquisition, critical revision, final approval. FM. Data analysis, interpretation, drafting, critical revision, final approval. AA P, AS Data acquisition, Data analysis, interpretation, final approval. SA. Data analysis, interpretation, critical revision, final approval. AA P, ASData analysis, drafting, interpretation, critical revision, final approval. SA, QF. Data analysis, critical revision, drafting, interpretation, final approval

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