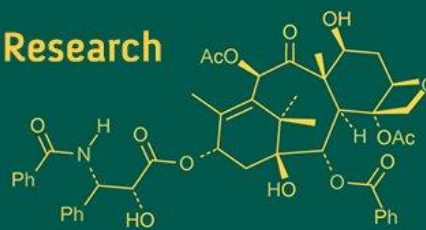
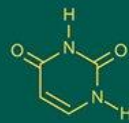


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Characterization of antimicrobial resistance pattern and genes in *Klebsiella* species isolated from domestic poultry and farm environment

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Abstract

Antimicrobial resistance (AMR) in *Klebsiella* species represents a growing threat to veterinary and human healthcare. This study aimed to analyze the antimicrobial resistance profiles by phenotypic and genotypic methods of seventeen isolates of *Klebsiella* isolated from cloacal swabs and nasal exudates from chickens as well as feed, water, soil, and litter were collected from poultry farms located in Mhow and Indore. The AMR pattern of isolated *Klebsiella* was determined using disc diffusion method. In vitro antimicrobial sensitivity testing revealed that all isolates were resistant to cefazolin and tigecycline. A high resistance rates among the isolates was also found against cefotaxime-clavulanic acid (94.11%) and ciprofloxacin (82.35%). PCR further confirmed presence of the bla_{TEM} and the bla_{SHV} genes in 94.11% and 88.23% isolates, respectively. A moderate correlation between the phenotypic resistance and the genotypic presence of specific resistance genes was recorded.

Keywords: *Klebsiella*; Poultry, Antimicrobial resistance; Multidrug resistant; Antibiotic Resistance genes

Introduction

The global antibiotic consumption in animals is estimated to be twice that of humans, and the improper and excessive use of these drugs in veterinary practices, both as therapeutic and growth promoter agents, has contributed significantly to the emergence of multidrug-resistant (MDR) microorganisms. This issue is particularly concerning in modernized intensive poultry farming systems, where large populations of birds are kept in close vicinity, creating an environment that fosters the spread of resistant bacteria (Chaudhry *et al.*, 2020) ^[1, 2].

Among the most problematic pathogens associated with antibiotic resistance is the ESKAPE group, which includes *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These pathogens are notorious for causing nosocomial infections and are known for their ability to "escape" the effects of antimicrobial treatments. *Klebsiella pneumoniae* is particularly concerning due to its role in severe infections, especially in immunocompromised and critically ill patients, and its increasing resistance to antibiotics (Singh, 2018) ^[3]. It also poses significant risks in animal populations, particularly poultry. The upsurge of antibiotic-resistant *Klebsiella pneumoniae* strains presents a major challenge in both veterinary and human medicine. Over the past few years, *K. pneumoniae* has developed resistance to a broad range of antibiotics, including β -lactams, aminoglycosides, carbapenems and fluoroquinolones. The primary mechanism behind this resistance is the production of extended-spectrum β -lactamases (ESBLs) and carbapenemases enzymes that break down antibiotics and render them ineffective. Carbapenems, which are often used as a last-resort treatment for severe infections, are becoming increasingly ineffective against *K. pneumoniae*, raising serious concerns about the future of treatment options (El-Sukhon, 2003) ^[4]. The overuse of antibiotics in both human medicine and animal agriculture has accelerated this trend, making it a pressing public health issue. Given the growing threat of MDR *K. pneumoniae*, understanding the sources and mechanisms of resistance in these bacteria is

crucial. This study aimed to achieve two primary objectives: 1) to analyze the antimicrobial resistance patterns exhibited by the *Klebsiella* isolates, and 2) to detect specific antimicrobial resistance genes (ARGs) in these isolates. These objectives were designed to provide insight into the genetic factors contributing to resistance and to assess the potential risk of transmission from animals to humans.

This research is critical for understanding the spread of MDR *Klebsiella* in domestic poultry and its surrounding environment. By identifying resistance patterns and detecting resistance genes, this study provides valuable information that can inform future strategies to control the spread of MDR bacteria in both veterinary and human healthcare settings. Understanding how *Klebsiella* species acquire resistance will aid in developing measures to mitigate the risk of antibiotic resistance spreading from animals to humans, ultimately contributing to better public health outcomes.

Materials and Methods

The study aimed to isolate *Klebsiella* species from poultry and the farm environment, assess antibiotic resistance patterns, and detect antimicrobial resistance genes (ARGs). A total of 150 samples were collected from various poultry farms around Mhow and Indore. Out of these 150 samples 17 came positive with phenotypic and genotypic methods. Antimicrobial sensitivity testing was performed using the disk diffusion method on Muller-Hinton agar (Bauer *et al.*, 1966)^[5]. Antibiotics used in the present study were: ampicillin/ sulbactam, cefotaxime/ clavulanic acid, cefazolin, ciprofloxacin, tetracycline, ertapenem, amoxycylav, aztreonam, ticarcillin, fosfomycin, chloramphenicol, gentamicin, ofloxacin, amikacin and tigecycline. The presence of ARGs was determined through PCR using specific primers for *bla TEM*, *bla SHV*, *bla DHAM*, *sul1*, *aadA* and *tetA* genes (Table 1), followed by gel electrophoresis to analyze the amplified DNA products. Thermocyclic conditions of each gene is given in table 2.

Table 1: Details of primers used for molecular characterization of ARGs.

Target gene	Target antibiotic	Primer Sequence	Product size (bp)	Reference
<i>bla TEM</i>	Beta lactam antimicrobial gene	F: TTGGGTGCACGAGTGGGTTA R: TAATTGTTGCCGGAAGCTA	506	Gundran <i>et al.</i> , 2020
<i>bla SHV</i>	Beta lactam antimicrobial gene	F:TCGGGCCGCGTAGGCATGAT R:AGCAGGGCGACAATCCCGCG	628	Gundran <i>et al.</i> , 2020
<i>tetA</i>	Tetracycline antimicrobial gene	F: GTGAAACCCAACATACCCC R: GAAGGCAAGCAGGATGTAG	887	Van <i>et al.</i> , 2008
<i>aadA</i>	Aminoglycoside antimicrobial gene	F:TGATTTGCTGGTTACGGTGAC R: CGCTATGTTCTCTTGCTTTTG	284	Van <i>et al.</i> , 2008
<i>Sul 1</i>	Sulphonamide antimicrobial gene	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	822	Shehata <i>et al.</i> , 2016
<i>bla DHAM</i>	Beta lactam antimicrobial gene	F:AACCTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC	405	Van <i>et al.</i> , 2008

Table 2: Thermocyclic conditions for the amplification of ARGs

Target ARG	Initial Denaturation	Denaturation	annealing	Extension	Final extension
Bla TEM	95°C 3 min	94°C 60 sec	56°C 30 sec	72°C 60 sec	72°C 7 min
Bla DHAM	95°C 15min	94°C 30 sec	54°C 30 sec	72°C 60 sec	72°C 10 min
Bla SHV	95°C 3 min	94°C 60 sec	64°C 30 sec	72°C 60 sec	72°C 7 min
aadA	95°C 15 min	94°C 30 sec	58°C 30 sec	72°C 60 sec	72°C 10 min
sul1	94°C 5 min	94°C 30 sec	56°C 30 sec	72°C 90 sec	72°C 10 min
tet A	94°C 5 min	94°C 30 sec	60°C 30 sec	72°C 60 sec	72°C 10 min

Results

Antimicrobial resistance pattern in *Klebsiella* isolates In vitro antibiotic sensitivity

In our study, all *Klebsiella* isolates were tested against 15 antibiotics using the disc diffusion method (Figure 1). Sensitivity results were interpreted according to the manufacturer's guidelines (Hi Media) and are detailed in Table 3 and Figure 2. The highest sensitivity was observed

for amikacin (82.35%), followed by ampicillin-sulbactam (58.82%), ticarcillinclavulanic acid, and ofloxacin (41.17% each). Notably, none of the isolates were sensitive to tigecycline, ciprofloxacin, cefazolin, or cefotaxime-clavulanic acid. The highest intermediate sensitivity was recorded for amoxycylav (52.94%) and gentamicin and ertapenem (47.05% each).

Table 3: Percentage sensitivity of *Klebsiella* isolates to different antimicrobial agents.

Sr. No.	Antimicrobial group	Antibiotics	Total number of isolates					
			Sensitive		Intermediate		Resistant	
			No	%	No.	%	No.	%
1	Aminoglycoside	Amikacin	14	82.35	1	5.88	2	11.76
2	Penicillin (Beta-lactam)	Amoxycylav	4	23.52	9	52.94	4	23.52
3	Aminopenicillin (Beta-lactam)	Ampicillin-Sulbactam	10	58.82	3	17.64	4	23.52
4	Monobactam (Beta- lactam)	Aztreonam	1	5.88	6	35.29	10	58.82
5	Cephalosporin (Beta- lactam)	Cefotaxim-clavulanic acid	0	0	1	5.88	16	94.11
6	Cephalosporin (Beta- lactam)	Cefazolin	0	0	0	0	17	100
7	Carbapenem (Beta lactam)	Ertapenem	2	11.76	8	47.05	7	41.17
8	Carboxypenicillin (Beta-lactam)	Ticarcillin- clavulanic acid	7	41.7	5	29.41	5	29.41
9	Phenicol	Chloramphenicol	4	23.52	7	41.17	6	35.29
10	Phosphonic acid	Fosfomycin	1	5.88	0	0	16	94.11
11	Aminoglycoside	Gentamicin	3	17.64	8	47.05	6	35.29
12	Quinolone	Ofloxacin	7	41.17	1	5.88	9	52.94
13	Tetracycline	Tetracycline	2	11.76	3	17.64	12	70.58
14	Floroquinilone	Ciprofloxacin	0	0	3	17.64	14	82.35
15	Glycylcyline	Tigecycline	0	0	0	0	17	100

In the study, resistance to ampicillin-sulbactam and amoxycylav was found to be 23.52% among *Klebsiella* isolates, while a higher resistance rate of 41.17% was observed for the carbapenem antibiotic ertapenem. Similarly, 23.52% of the isolates were resistant to the monobactam aztreonam. Notably, cefotaxime-clavulanic acid, a cephalosporin, showed no sensitivity towards the tested isolates. Ciprofloxacin, a third-generation fluoroquinolone, had a high resistance rate of 82.35%, particularly in ESBL-positive strains, a trend consistent with our findings. Additionally, 70.58% of *Klebsiella* strains were resistant to tetracyclines, while resistance to

aminoglycosides, such as amikacin and gentamicin, was 11.76% and 35.29%, respectively. Resistance to chloramphenicol was observed in 35.29% of the isolates, and importantly, all isolates were 100% resistant to tigecycline. In the present study six isolates of *Klebsiella* spp were found to be multiple drug resistant (MDR), as they showed resistance to at least one antimicrobial agent (Table 4) in three antimicrobial categories as per Sweeney *et al.* (2018)^[6]. Out of 6 MDR strains, 3 were *K. pneumoniae*, which suggests this bacteria is an emerging multidrug-resistant (MDR) pathogen.

Table 4: Multiple drug resistance in *Klebsiella* strains

Isolate No.	Antibiotics															Remarks
	AK	AMC	A/S	AT	CEC	CZ	C	CIP	ETP	FO	GEN	OF	TE	TCC	TGC	
1	S	S	R	I	R	R	S	R	S	R	I	R	R	S	R	-
2	S	I	S	I	R	R	S	R	I	R	R	R	R	S	R	-
3	R	S	S	S	I	R	R	I	I	R	S	S	R	S	R	-
4	S	R	S	R	R	R	I	R	R	S	R	R	R	S	R	-
5	S	I	S	R	R	R	R	R	R	R	R	R	R	R	R	MDR
6	S	S	R	I	R	R	I	R	I	R	R	R	R	S	R	-
7	S	I	S	R	R	R	I	I	I	R	I	S	S	S	R	-
8	S	R	I	R	R	R	R	R	R	R	R	R	R	R	R	MDR
9	S	I	S	R	R	R	R	R	R	R	I	R	R	R	R	MDR
10	S	I	S	R	R	R	I	I	I	R	I	S	S	S	R	-
11	S	I	S	I	R	R	S	R	I	R	I	S	I	I	R	-
12	S	I	S	R	R	R	R	R	R	R	R	S	R	I	R	MDR
13	I	R	S	R	R	R	I	R	R	R	I	S	I	R	R	-
14	S	R	I	I	R	R	I	R	I	R	I	I	R	I	R	MDR
15	S	I	R	R	R	R	R	R	R	R	S	R	R	R	R	MDR
16	S	I	R	I	R	R	S	R	I	R	I	S	R	I	R	-
17	R	S	I	R	R	R	I	R	S	R	S	R	I	I	R	-

Detection and prevalence of antimicrobial resistance genes in *Klebsiella* isolates

PCR analysis of DNA from 17 *Klebsiella* isolates identified the bla TEM gene in 16 isolates (94.11%) through a 506 bp amplicon (Figure 3), while the bla SHV gene was detected in 15 isolates (88.23%) with a 628 bp product (Figure 4). The bla DHAM gene, marked by a 405 bp amplicon, was present in only 2 isolates (11.78%) (Figure 5). The sul1 gene was found in 3 isolates (17.6%) with an 822 bp amplicon

(Figure 6). Additionally, the aadA gene (Figure 7) associated with aminoglycoside resistance, was present in 7 isolates (41.17%) with a 284 bp product, and the tetA gene, linked to tetracycline resistance, and was also detected in 7 isolates (41.17%) with an 887 bp product (Figure 8). The prevalence of antimicrobial resistance genes (ARGs) is depicted in the form of a pie chart in Figure 9 and table 5 summarizes the different sample sources in which these genes were detected.

Table 5: Prevalence of different ARGs in *Klebsiella* isolates

Isolate no.	Source of sample	Species	Prevalence of ARGs in <i>Klebsiella</i> isolates %					
			Sul1	Tet A	aadA	bla SHV	bla DHAM	bla TEM
1	Drinking water	K.p	+	+	-	-	+	+
2	Feed sample	K.p	-	+	+	+	-	+
3	Cloacal swab	K.p	-	+	+	+	-	+
4	Cloacal swab	K.p	-	+	+	+	-	+
5	Feed sample	Not identified	-	+	+	+	-	+
6	Cloacal swab	K.p	-	+	+	+	-	+
7	Cloacal swab	K.p	-	-	+	+	-	+
8	Cloacal swab	K.p	+	-	-	+	-	+
9	Cloacal swab	K.p	+	-	+	+	-	+
10	Cloacal swab	K.p	-	+	+	+	-	+
11	Cloacal swab	K.p	-	-	-	+	-	+
12	Cloacal swab	K.p	-	-	-	-	-	+
13	Cloacal swab	K.p	-	-	-	+	-	+
14	Soil sample	Not identified	-	+	-	+	-	+
15	Litter material	Not identified	-	-	+	+	+	+
16	Drinking water	Not identified	-	-	-	+	-	+
17	Soil sample	Not identified	-	-	-	+	-	-
Prevalence OF ARGs (%)			17.64	41.17	41.17	88.23	11.76	94.11

Co-relation between phenotypic and genotypic resistance

A moderate correlation was observed between phenotypic resistance (AST) and genotypic resistance (ARG) in *Klebsiella* strains in this study. The phenotypic resistance of 82.30% to amoxycylav and ampicillin aligned with the detection of *bla TEM* and *bla SHV* genes in 94.11% and 88.23% of isolates, respectively. Similarly, phenotypic resistance to aminoglycosides (amikacin and gentamicin) at 47% correlated with the presence of the *aadA* gene in 41.17% of isolates.

Discussion

Increasing antimicrobial resistance is a significant public health issue, with scientists predicting it could lead to the next pandemic. The treatment of infections caused by Gram-negative bacteria, such as *Klebsiella*, poses a major challenge, especially given the indiscriminate use of antibiotics in livestock and poultry industries, which contributes to the emergence of drug-resistant bacteria (Exner *et al.*, 2017) [17]. In our study, all *Klebsiella* isolates were tested for susceptibility to 15 antibiotics using the disc diffusion method. The highest sensitivity was observed for amikacin (82.35%), while no isolates were sensitive to tigecycline, ciprofloxacin, cefazolin, or cefotaxime/clavulanic acid. Our findings align with previous studies by Younis *et al.* (2016) [8], Akinbami *et al.* (2018) [9], and others, which also reported *Klebsiella* strains sensitive to amikacin but resistant to ampicillin, amoxycylav, aztreonam, and cefotaxime. However, our study contrasts with Diagbougba *et al.* (2016) [10], who reported high sensitivity to fosfomycin, a finding not supported by our results. The importance of conducting antibiotic sensitivity testing (AST) prior to treating *Klebsiella* infections is underscored by the potential for changes in drug sensitivity over time, as highlighted by Aly *et al.* (2014) [11].

Antimicrobial resistance pattern in *Klebsiella* isolates

Antimicrobial resistance has emerged in zoonotic enteropathogens, commensal bacteria, and animal pathogens, with varying prevalence (Carattoli, 2008) [12]. Factors influencing antibiotic resistance include bacterial resistosomes, misuse of antibiotics as therapeutic or prophylactic agents, improper dosing, and the use of

antibiotics as growth promoters in livestock (Watkins and Bonomo, 2016; Van Boeckel *et al.*, 2015) [13, 14]. In our study, the high degree of resistance observed may be due to the misuse of antibiotics in poultry feed, as also noted by El Tawab *et al.* (2018) [15]. The prolonged use of low-dose antibiotics can lead to the survival of resistant strains, which eventually dominate the population, contributing to the widespread distribution of antimicrobial resistance (Woodford *et al.*, 2011) [16].

Resistance to beta lactam antibiotics

Extended spectrum beta-lactam (ESBL) antibiotics, including penicillins, cephalosporins, cephamycins, carbapenems, and monobactams, are widely used to treat serious Gram-negative infections. However, bacterial resistance has emerged due to the production of ESBL enzymes, which deactivate these antibiotics by hydrolyzing the betalactam ring (Bajpai *et al.*, 2017) [17]. In our study, resistance to ampicillin/sulbactam and amoxycylav was observed at 23.52%, which is lower than rates reported by Younis *et al.* (2016) [8] and others. *Klebsiella* species naturally resist penicillins due to low production of a class A chromosomal β -lactamase, but resistance can increase when ESBL genes are acquired (Brise and Duijkeren, 2005) [18]. Ertapenem resistance was high at 41.17%, aligning with Hamza *et al.* (2016) [19], highlighting a growing concern of carbapenem resistance even though these drugs are not typically used in poultry. Aztreonam resistance was 23.52%, similar to findings by Tewari *et al.* (2019) [20]. No sensitivity to cefotaxime/clavulanic acid was observed, consistent with previous studies (Younis *et al.*, 2016; Elgaos *et al.*, 2019) [8, 21]. Increasing resistance to cefotaxime, a critical third-generation cephalosporin, may be due to its frequent use in veterinary medicine, as noted in recent reports.

Resistance to quinilones and fluoroquinilones

Fluoroquinolones are critical antibiotics for managing bacterial infections (Cattoir and Nordmann, 2009) [22]. In our study, resistance to ciprofloxacin, a third-generation fluoroquinolone, was observed at 82.35%, which aligns with findings by Diagbougba *et al.* (2016) [10], Younis *et al.* (2016) [8], and others, although Elgaos *et al.* (2019) [21] reported a much lower resistance rate of 20%. In India, resistance to

fluoroquinolones among *Klebsiella* spp. has risen from 57% to 73% (CDDEP, 2015). This resistance is often due to chromosomal mutations in quinolone resistance-determining regions (QRDR) and plasmid-mediated mechanisms (Correia *et al.*, 2017) ^[23], with higher resistance observed in ESBL-positive strains, as seen in our study. The increasing use of fluoroquinolones in poultry farming, including as growth promoters, may be contributing to the development of resistance, a trend confirmed by veterinarians in the study area (Mahanti *et al.*, 2018) ^[24].

- **Resistance to Tetracyclines:** In our study, 70.58% of *Klebsiella* strains were resistant to tetracyclines, consistent with findings by Bokaeian *et al.* (2014) ^[25] and Kashefieh *et al.* (2021) ^[26]. Tetracycline resistance is often mediated by ribosomal protection, efflux pumps, or chemical modifications (Moradigaravand *et al.*, 2017) ^[27]. The widespread use of tetracycline in poultry farms, including in India, due to its broad-spectrum efficacy and low cost, may contribute to this resistance (Michalova and Schlegelova, 2004) ^[28].
- **Resistance to Aminoglycosides:** Resistance to aminoglycosides like amikacin and gentamicin was observed at 11.76% and 35.29%, respectively, aligning with the findings of Mokhtari *et al.* (2018) ^[29] and Kashefieh *et al.* (2021) ^[26]. Aminoglycoside resistance

mechanisms include enzymatic modification and changes in membrane permeability (El-Badawy *et al.*, 2017) ^[30]. These drugs are less commonly used in farming due to their cost, which may explain the lower resistance observed (Sidibe *et al.*, 2019).

- **Resistance to Phenolics:** Chloramphenicol resistance in *Klebsiella* was 35.29% in our study, similar to Younis *et al.* (2016) ^[8]. Chloramphenicol resistance is often due to acetylation by chloramphenicol acetyltransferases (Schwarz *et al.*, 2004) ^[31], and its use has declined due to associated side effects.
- **Resistance to Phosphonic Acid:** Fosfomycin resistance was very high at 94.11% in our *Klebsiella* isolates, a concerning finding given its limited use in veterinary practice. This high resistance may be due to horizontal gene transfer from other bacteria (Akinbami *et al.*, 2018) ^[9].
- **Resistance to Glycylcyclines:** All *Klebsiella* isolates in our study were 100% resistant to tigecycline, which contrasts with the 9% resistance reported by Kashefieh *et al.* (2021) ^[26]. Tigecycline resistance in *Klebsiella* is often due to efflux pumps, particularly RND-type transporters (Sun *et al.*, 2013) ^[32].

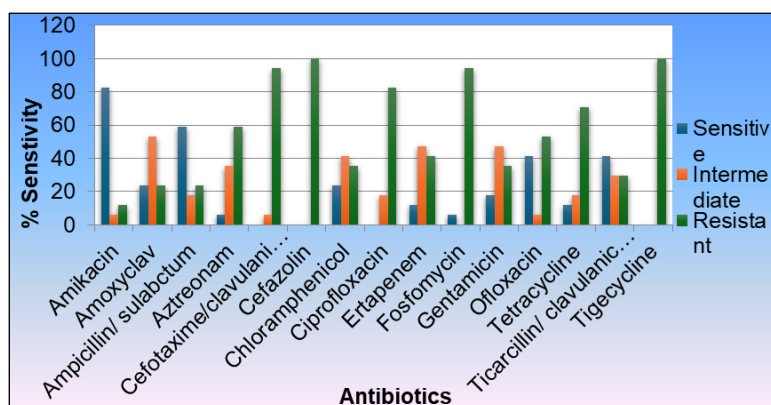


Fig 1: Percentage sensitivity of *Klebsiella* to different antimicrobial agents

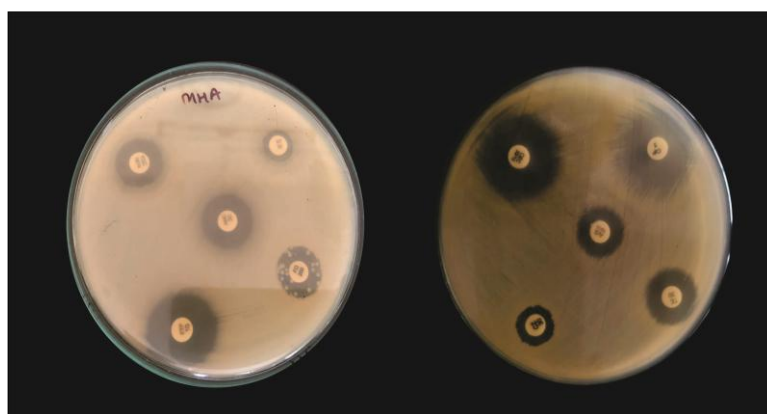


Fig 2: Antibiotic sensitivity test.

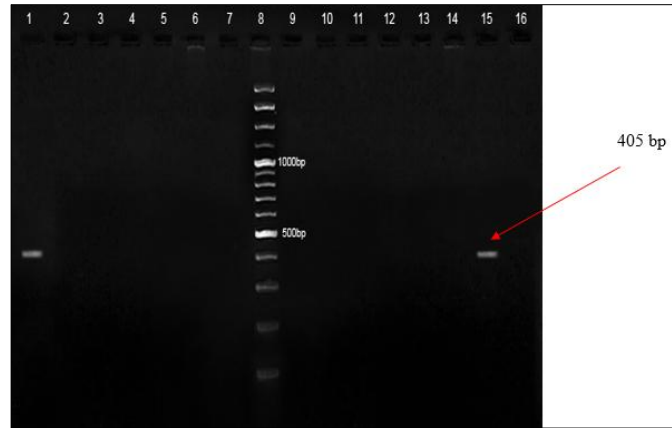


Fig 3: Agarose gel electrophoresis showing amplified product (405bp) of *bla* DHAM gene

- **Lane 1, 15:** Positive samples
- **Lane 2-7, 9-14:** Negative samples
- **Lane 8:** Ladder (100bp)

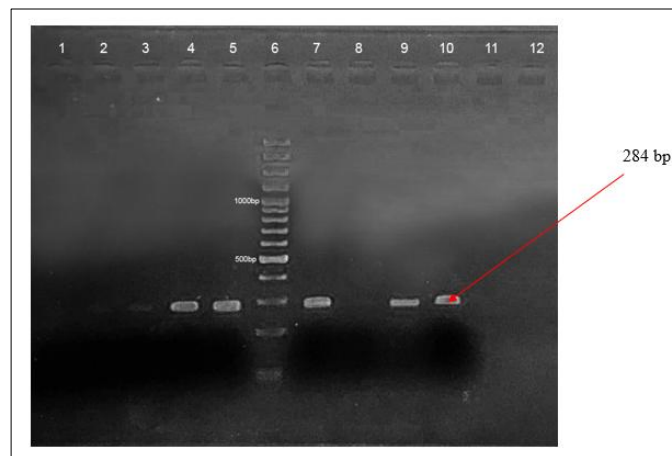


Fig 4: Agarose gel electrophoresis showing amplified product (284bp) of *aadA* gene

- **Lane 2, 3, 5, 7, 9, 10:** Positive samples
- **Lane 1, 8, 12:** Negative sample
- **Lane 6:** Ladder (100bp)

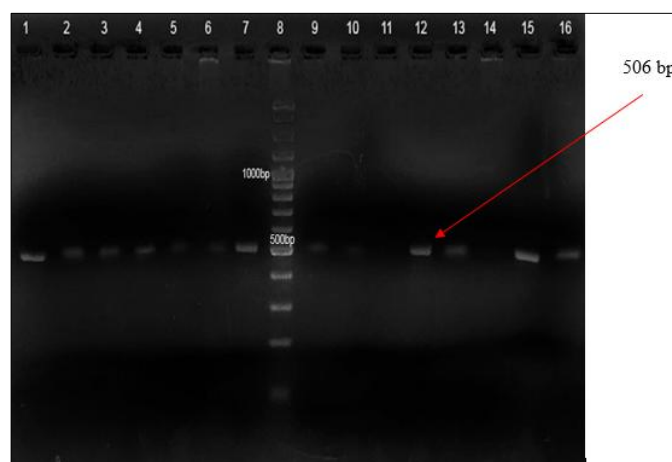


Fig 5: Agarose gel electrophoresis showing amplified product (506bp) of *bla* TEM gene

- **Lane 1-5, 7, 9, 10, 12, 13, 14, 15, 16:** Positive samples
- **Lane 6:** Negative sample
- **Lane 8:** Ladder (100bp)

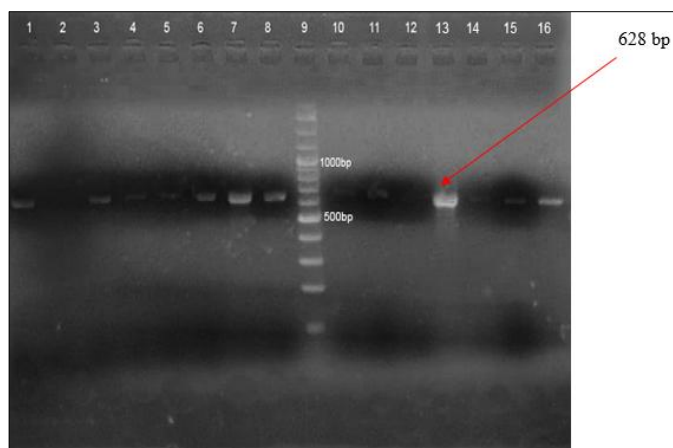


Fig 6: Agarose gel electrophoresis showing amplified product (628bp) of *bla* SHV gene

- **Lane 1, 3-8, 11, 13-16:** Positive samples
- **Lane 9:** Ladder (100bp)

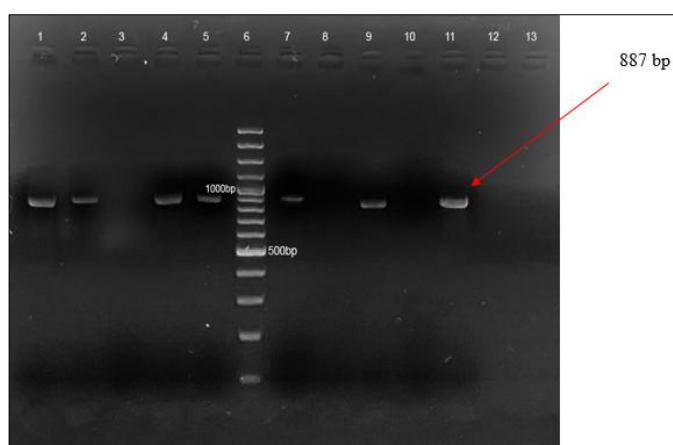


Fig 7: Agarose gel electrophoresis showing amplified product (887bp) of *tetA* gene

- **Lane 1-5, 7, 10, 11:** Positive samples
- **Lane 8:** Negative samples
- **Lane 6:** Ladder (100bp)

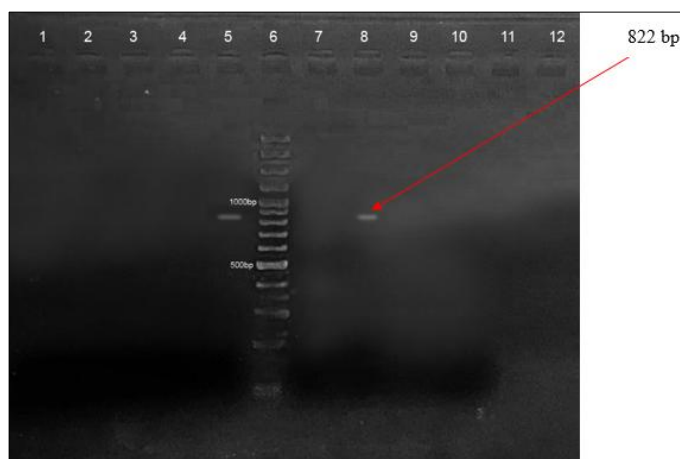


Fig 8: Agarose gel electrophoresis showing amplified product (822bp) of *sul1* gene

- **Lane 5, 8:** Positive samples
- **Lane 1-4, 7, 9-12:** Negative sample
- **Lane 6:** Ladder (100bp)

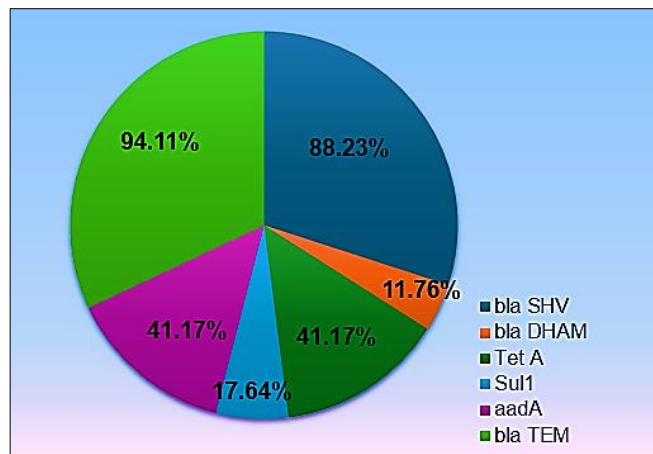


Fig 9: Prevalence of antimicrobial resistance genes in *Klebsiella* isolates

Simultaneous and multiple drug resistance

All 17 isolates showed resistance to 5 to 14 antibiotics, with 64.70% (11 isolates) resistant to 8 to 13 antibiotics, although none were resistant to all 15. These results align with Brower *et al.* (2017) [33]. Six isolates of *Klebsiella* spp. were identified as multidrug-resistant (MDR), showing resistance to at least one agent in three different antimicrobial categories, according to Sweeney *et al.* (2018) [6]. Notably, three of these MDR strains were *K. pneumoniae*, highlighting its role as an emerging MDR pathogen, consistent with findings by Navon Venezia *et al.* (2017) [34] and others. This emphasizes *K. pneumoniae* as a significant contributor to antibiotic resistance, classified as an ESKAPE organism (Boucher *et al.*, 2009) [35].

Detection of antimicrobial resistance genes in *Klebsiella* isolates

Under antibiotic pressure, *Klebsiella* rapidly accumulates antibiotic resistance genes (ARGs) through mutations and acquisition of plasmids and mobile genetic elements. This resistome, the collection of all resistance genes, is often carried on self-transferable plasmids within *K. pneumoniae*, contributing to the development of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains (Navon-Venezia *et al.*, 2017) [34]. In our study, we detected the *bla TEM* and *bla SHV* genes in 94.11% and 88.23% of *Klebsiella* isolates, respectively, with *bla TEM* being the most prevalent, aligning with findings from previous studies (Fortini *et al.*, 2011; Bajpai *et al.*, 2017) [36, 17]. Additionally, the *aadA* and *tetA* genes, responsible for aminoglycoside and tetracycline resistance, were present in 41.17% of isolates, consistent with other reports (Kashefieh *et al.*, 2021) [26]. The emergence of these resistance genes highlights the significant role of *K. pneumoniae* in spreading antibiotic resistance, especially in settings with high antibiotic use.

The rise of antibiotic resistance in livestock production is a critical issue, with improper antibiotic use closely linked to the development of AMR in humans (Ozawa *et al.*, 2012) [37]. This study showed a moderate correlation between phenotypic and genotypic resistance in *Klebsiella* strains, with phenotypic resistance to amoxycylav and ampicillin (82.30%) aligning with the presence of *bla TEM* and *bla SHV* genes (94.11% and 88.23%, respectively). Resistance to aminoglycosides (47%) also correlated with the *aadA* gene (41.17%). Effective surveillance and characterization

of antimicrobial-resistant bacteria in livestock and poultry are essential (Tewari *et al.*, 2019) [20].

Conclusion

This study highlights the significant presence of antimicrobial-resistant *Klebsiella* species in poultry farms, with a 11.3% prevalence across samples and the highest concentration in farm soil (50.5%). Notably, *Klebsiella pneumoniae* was identified as the most pathogenic, found in 8% of samples. High resistance rates to ciprofloxacin (82.35%) and tigecycline (100%) were observed, with *bla TEM* and *bla SHV* genes detected in 94.11% and 88.23% of isolates, respectively. The findings emphasize the need for stringent antibiotic management and continuous monitoring to prevent the spread of multidrug-resistant bacteria, protecting both veterinary and human health.

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